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FILE 'USPAT' ENTERED AT 11:29:16 ON 24 APR 96
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  *                W E L C O M E   T O   T H E                          *
  *          U. S.   P A T E N T   T E X T   F I L E                    *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
=> s nuclear localization and transfection
         49871 NUCLEAR
          5658 LOCALIZATION
            39 NUCLEAR LOCALIZATION
                 (NUCLEAR(W)LOCALIZATION)
          1723 TRANSFECTION
L1          18 NUCLEAR LOCALIZATION AND TRANSFECTION
=> d 1-18 kwic

US PAT NO:     5,496,731 [IMAGE AVAILABLE]              L1: 1 of 18

SUMMARY:

BSUM(71)

 In . . . proliferation of those cells. The expression vector is
inserted into the abnormally proliferating cells by viral infection or
transduction, liposome-mediated **transfection**, polybrene-mediated
**transfection**, CaPO4 mediated **transfection** and electroporation.
The treatment is repeated as needed.

DRAWING DESC:

DRWD(10)

 FIG. . . . bladder tumor cells were transfected in multiple dishes
with either p110.sup.RB (p.beta.A-f-RB33) or p94.sup.RB (p.beta.A-s-RB34)
expression plasmids. Twenty-four hours after **transfection** the cells
were labeled with [.sup.35 S]-methionine and chased with excess unlabeled
methionine for 0, 6, 12 and 24 hours,. . .

DETDESC:

DETD(5)

 The effects of **transfection** by either first or second in-frame AUG
codon-initiated RB protein expression plasmid were compared on a number
of well known. . .

DETDESC:
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DETD(29)

The . . . a plasmid or viral expression vector. A plasmid expression vector may be introduced into a tumor cell by calcium phosphate **transfection**, liposome (for example, LIPOFECTIN)-mediated **transfection**, DEAE Dextran-mediated **transfection**, polybrene-mediated **transfection**, electroporation and any other method of introducing DNA into a cell.

DETDESC:

DETD(48)

The . . . is administered in a composition comprising the vector together with a carrier or vehicle suitable for maintaining the transduction or **transfection** efficiency of the chosen vector and promoting a safe infusion. Such a carrier may be a pH balanced physiological buffer,. . .

DETDESC:

DETD(61)

Since non-functional mutations of the retinoblastoma protein are characterized by defects in phosphorylation, viral oncoprotein association and **nuclear** **localization** (Templeton et al., 1991, Proc. Natl. Acad. Sci., USA, 88:3033-3037), the functional aspects of the artificial p94.sup.RB protein were studied. . .

DETDESC:

DETD(100)

For . . . DNA p.beta.A-s-RB34 (or pCMV-s-RB42) via LIPOFECTIN reagent (GIBCO BRL Life Technologies, Inc. Gaithersberg, Md.). Similar results have been obtained from **transfection** using calcium phosphate or electroporation methods.

DETDESC:

DETD(101)

The following procedures for **transfection** using LIPOFECTIN were modified from the manufacturer's specifications. Tumor cells were seeded in 100-mm dishes in appropriate growth medium supplemented. . . complex. While the lipid-DNA complex was forming, the cells were washed twice with 6 ml of serum-free medium. For each **transfection**, 6 ml of

serum-free medium were added to each polystyrene tube containing the lipid-DNA complex. The solution was mixed gently,. . .

DETDESC:

DETD(105)

TABLE 2

Immunocytochemical Staining and [.sup.3 H] Thymidine Incorporation of RB-Defective Tumor Cells Following **Transfection** With p94.sup.RB or p110.sup.RB Expression Plasmids

Recipient Cells	Promoter	Protein Expressed	Cells Incorporating [.sup.3 H] Thymidine RB+	RB-

. . .

DETDESC:

DETD(107)

Approximately 48 hours after **transfection** the tumor cells were replated at a density of 10.sup.5 cells per 100 mm dish with selected medium containing G418. . .

DETDESC:

DETD(108)

Furthermore, . . . p94.sup.RB did suppress tumor cell growth. In contrast, 7 of 48 cell lines (approximately 15%) derived from tumor cells after **transfection** with the p110.sup.RB plasmid DNA were found to express the p110.sup.RB protein. This percentage was consistent with results expected in. . .

DETDESC:

DETD(111)

The HTB9 transfectants were also immunostained with MAb-1 anti-RB monoclonal antibody about 24 hours after **transfection**. The staining results are illustrated in FIG. 8.

DETDESC:

DETD(114)

Two . . . of G418-resistant colonies formed after treated with the plasmid victor pCMV-s-RB42 expressing p94.sup.RB, while no such effect was observed by **transfection** with the pCMV-f-RB35 plasmid (expressing p110.sup.RB protein). The difference was statistically significant (the two-tailed P values were less than 0.03. . .

DETDESC:

DETD(118)

The . . . transfected in multiple dishes with either p110.sup.RB (FIG. 9, left) or p94.sup.RB (FIG. 9, right) expression plasmids. Twenty-four hours after **transfection** the cells were labeled with [.sup.35 S]-methionine and chased with excess unlabeled methionine for 0, 6, 12 and 24 hours,. . .

DETDESC:

DETD(120)

The . . . and p.beta.A-f-RB33 (expressing p110.sup.RB, Section 4.3.5) or p.beta.A-s-RB34 (expressing p94.sup.RB Section 4.3.4) plasmid transfected 5637 cells approximately 24 hours after **transfection**. The basic protocal for Western blot analysis was described in Xu, H-J., et al., 1989, Oncogene, 4:807-812. Each lane was. . .

US PAT NO: 5,470,736 [IMAGE AVAILABLE] L1: 2 of 18

SUMMARY:

BSUM(6)

Several . . . an alternatively spliced c-myb mRNA encodes a truncated form of the c-myb p75 which includes the DNA binding region and **nuclear** **localization** signal present in c-myb protein, but lacks regulatory regions required for transcriptional activation (Weber, et al., Science, 249:1291, 1990). The. . .

DETDESC:

DETD(19)

When the host is a eukaryote, such methods of **transfection** of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, insertion of a plasmid encased in liposomes, or. . .

DETDESC:

DETD(28)

Alternatively, . . . cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate **transfection**. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral. . .

DETDESC:

DETD(69)

FosB2 . . . Briefly, embryonic carcinoma F9 cells were plated in 10 cm dishes at a density of 5.times.10.sup.6 cells/plate 24 hours before **transfection**. **Transfection** protocol was as described (Chen, et al., Mol. Cell. Biol., 7:2745-2752, 1987) and .beta.-galactosidase activity was assayed by the CNPG. . . the reporter construct 5XTRECAT and 2 .mu.g of pBAG (a .beta.-galactosidase expression plasmid, which served as an internal control of **transfection** efficiency) were cotransfected into F9 cells with various combinations of expression plasmids.

DETDESC:

DETD(70)

For . . . reaction products were analyzed on TLC plates as described (Gorman, et al., Mol. Cell. Biol., 2:1044-1051, 1982). Forty-eight hours after **transfection**, CAT activity was measured. All CAT activity assays were standardized with .beta.-galactosidase activity. Fold induction was standardized with the control. . .

DETDESC:

DETD(75)

208F . . . along with different amounts of a FosB2 expression plasmid (from 0-16 .mu.g); the total amount of DNA used in each **transfection** was kept constant by varying the amount of carrier DNA. Focus assays were performed as previously described (Miller, et al., Cell, 36:51-60, 1984). Each **transfection** was plated in duplicate. Foci were counted 12 days after **transfection** for v-Fos transfected cells, and 17 days after **transfection** for c-Fos and FosB transfected cells. FosB2 interfered with both the transcriptional transactivation and transformation potential of c-Fos and FosB,. . .

DETDESC:

DETD(87)

Ten . . . lines known in the art can alternately be used. The medium is changed 24 hours later and 48 hours after **transfection**, the culture medium is harvested and used to infect the amphotropic packaging cell line .PSI.-CRIP, for example, in the presence. . .

US PAT NO: 5,468,624 [IMAGE AVAILABLE] L1: 3 of 18

SUMMARY:

BSUM(3)

The . . . the steroid binding domain. This domain binds glucocorticoid to activate the receptor. This region of the receptor also has the **nuclear** **localization** signal. Deletion of this carboxyl terminal end results in a receptor that is constitutively active for gene induction (up to. . .

DRAWING DESC:

DRWD(2)

FIG. 1 shows the results of **transfection** of ICR 27 cells with GR constructs. ICR 27 cells were transfected with 4 different GR constructs, namely holo GR,. . . for cell kill in the absence (-) or presence (+) of 10.sup.-6 M dex for up to 96 hours after **transfection**. The numbers above the boxes correspond to the amino acid position in the protein sequence of the steroid receptor. The. . . domain, respectively. The percentage reduction in viable cell number, both in the absence (-) or presence (+) of dex, following **transfection** of ICR 27 cells with these steroid receptor constructs is indicated. Superscript "a" indicates results for cell kill that are. . .

DRAWING DESC:

DRWD(3)

FIGS. . . . cell that is not transfected and on the right is a transfected cell. For all three cell lines, efficiency of **transfection** was about 40%.

DRAWING DESC:

DRWD(4)

FIG. . . . a control to measure percentage cell lysis or reduction in viable cell number, in the absence (-) of dex following **transfection**. Values given represent maximum kill observed. Maximum kill with or without dex occurred between 6-24 hr. Each **transfection** was done in triplicate.

DETDESC:

DETD(4)

Tests . . . in the range of 39%-51%, which is in agreement with the results of cell kill. Successive transfections of cells surviving **transfection** with glucocorticoid receptor constructs resulted in similar efficiencies of cell kill each time, confirming that electroporation was not merely eliminating. . . took up the DNA. By blocking de novo protein and RNA synthesis, the onset of cell kill was arrested following **transfection** with an otherwise highly lethal constitutive receptor construct. Once the drug was removed and protein and RNA synthesis allowed to. . .

DETDESC:

DETD(19)

The . . . (BioRad Laboratories, Richmond, Calif.) at 200 V, 500 .mu.F capacitance. The time constants or pulse time was recorded for each **transfection**--it ranged between 12 and 15 milliseconds. Five minutes after electroporating, the cells were resuspended to 4.times.10.sup.5 cells/ml in RPMI 1640. . .

DETDESC:

DETD(20)

In experiments to verify the consistency of **transfection** efficiency, ICR 27 cells were electroporated three times in succession with glucocorticoid receptor constructs 465* and PRSHGRA. After each **transfection**, cell counts were determined soon after as well as 6, 12 and 24 hours after **transfection**. Cells were allowed to recover for 48 hours before being subject to a repeat pulse in the series of transfections.

DETDESC:

DETD(22)

At 24 hours after **transfection**, cells were resuspended to 2.times.10.sup.5 cells/ml in RPMI 1640 with 5% fetal bovine serum. When appropriate, dexamethasone (Sigma, St. Louis,. . .

DETDESC:

DETD(25)
Determining the Efficiency of **Transfection**

DETDESC:

DETD(26)

In separate **transfection** experiments, ICR 27 cells were transfected by the protocol as described earlier with 15 .mu.g of P.sup.32 -labeled DNA, specifically PRSHGRA, 465* and 398-465*. Each **transfection** was done in duplicate. To label the DNA, the plasmids were digested with KpnI (to generate a 3' overhang), incubated. . . silver grains (above background i.e. >5 grains/cell) was recorded. Between 50-100 consecutive cells were counted per slide (4 slides per **transfection**).

DETDESC:

DETD(28)

Preliminary . . . synthesis but not kill cells. ICR 27 cells were treated with cycloheximide or ethanol vehicle before electroporation and subsequently to **transfection** with 465* and pRShGR.alpha.. Cell counts and viability were recorded 15 minutes after, as well as 6, 12 and 19. . .

DETDESC:

DETD(29)

In . . . 30 minutes prior to electroporation. The cells were washed with PBS and transfected. Cycloheximide or ethanol was readded subsequent to **transfection**. At 30 minutes and 4-6 hours after **transfection** RNA and protein synthesis block was measured by incubating in the presence of the labeled precursors, TCA precipitating the products. . .

DETDESC:

DETD(31)

Transfection of holo glucocorticoid receptor into

glucocorticoid-resistant ICR 27 cells could restore cell lysis on addition of 10.sup.-6 M dexamethasone (27,28,30). Since these were transient **transfection** assays, the extent of lysis was not 100%, but averaged 26.+-.4% in 23 assays, each done in triplicate. The holoreceptor. . .

DETDESC:

DETD(33)

A . . . construction is described earlier, was used to transfect ICR 27 cells as shown in FIG. 1. Within 6-24 hours of **transfection** and in the absence of dexamethasone 28% of the cells were lysed--an extent comparable to that evoked by the holoreceptor and steroid 48-96 hours after **transfection**. Thus, a sequence which spans less than 100 amino acids is responsible for the constitutive lethality of the receptor. Although. . .

DETDESC:

DETD(34)

The following fragments or mutations of the GR gene were inactive for cell lysis upon **transfection**: .DELTA.420-451 (deletion of the first zinc finger); .DELTA.450-487 (deletion of the second zinc finger); .DELTA.428-490 (deletion of the entire DNA. . .

DETDESC:

DETD(38)

Table 2 indicates the maximum percentage cell kill along with standard deviation values, seen in different cell lines, either after **transfection** with GR constructs 465* without 1 .mu.M dex (in the second column) or the entire GR in the presence of. . .

DETDESC:

DETD(40)

The efficiency of **transfection** was determined in several experiments as all the assays were transient and no reporter gene had been co-transfected to serve as a control. ICR 27 cells were transfected with P.sup.32 -labeled constructs pRShGR.alpha., 465* and 398-465* in two separate **transfection** experiments. The labeled DNA in the cells was visualized by autoradiography. In the transfections with the holoreceptor between 40%-50% of. . . of cell kill varied between 22%-39% (27) for

these three glucocorticoid receptor constructs, which correlates well with the results of **transfection** efficiency, since it would be expected that a proportion of cells transfected would retain and express sufficient DNA to show. . .

DETDESC:

DETD(42)

To determine if similar fractions of cells would be killed if the cells surviving **transfection** are electroporated repeatedly, ICR 27 cells were electroporated three times in succession with 465* or the holoreceptor (as a control) as shown in FIG. 3. After each **transfection**, cells were allowed to recover for 48 hours before being subjected to a repeat pulse. Similar fractions of cells were. . .

DETDESC:

DETD(44)

Cycloheximide . . . protein synthesis and/or RNA synthesis to determine if the lethal effects of 465* were specifically due to a product of **transfection** rather than **transfection** of the DNA itself into cells. The cell lethality kinetics of 465* are quick and therefore compatible with the maximum. . .

DETDESC:

DETD(46)

The . . . 21 missense amino acids and stops. It has some sequence important for binding GREs but lacks signals for transcriptional activation, **nuclear** **localization**, steroid binding, and most sites for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of **transfection**. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . .

DETDESC:

DETD(47)

The efficiency of transient **transfection** assays was consistently in the range of 39%-51% irrespective of the gene construct used. These values are slightly higher than. . .

DETDESC:

DETD(48)

By repeatedly transfecting cells surviving **transfection** (three times in succession) similar killing efficiency was obtained and rules out the possibility that the transfections were wiping out. . .

US PAT NO: 5,449,755 [IMAGE AVAILABLE] L1: 4 of 18

DETDESC:

DETD(3)

The . . . translation of a cyclin E mRNA. Antisense nucleic acids may be encoded within a host cell, e.g., following transduction or **transfection** of the cell with a vector DNA or RNA sequence encoding an antisense nucleic acid, or, alternatively, the antisense nucleic. . .

DETDESC:

DETD(13)

Aspects . . . resistance markers, or markers satisfying the ,growth requirements of the cell. It will also be appreciated that in certain cells **transfection** or transduction with cyclin E nucleic acid will provide a selective proliferative/growth advantage that will serve as a type of. . .

DETDESC:

DETD(162)

Booher, . . . E., Hyams, J. S., and Beach, D. H. (1989). The fission yeast cdc2/cdc13/suc1 protein kinase: regulation of catalytic activity and **nuclear** **localization**. Cell 58, 485-497.

US PAT NO: 5,403,712 [IMAGE AVAILABLE] L1: 5 of 18

DETDESC:

DETD(10)

Alternatively, . . . membrane protein which results in transport of the peptide into the cell. For localization to the nucleus one may use **nuclear** **localization** sequences such as those defined for the glucocorticoid receptor or SV-40 large T antigen.

DETDESC:

DETD(44)

Transient **Transfection** Studies

DETDESC:

DETD(46)

For . . . duplicate or triplicate, and 2 .mu.g of the RSV-luciferase plasmid was included in each sample as an internal control for **transfection** efficiency. The total amount of DNA transfected into each sample of a given experiment was held constant by including an. . . an insert. CAT (Gorman et al., 1982) and luciferase (de Wet et al., 1987) activity were measured 36-48 hours after **transfection**. CAT assays were quantitated on an AMBIS radioactivity scanner, and the amount of CAT activity in each sample was corrected for **transfection** efficiency based on the results of the luciferase assay.

DETDESC:

DETD(51)

FIG. . . . bars) or presence (shaded bars) of DCoH expression vector. Cells were assayed for CAT activity 36 to 48 hours after **transfection**. Results are presented as the averages of duplicate measurements in a representative experiment and are corrected for **transfection** efficiency (Horton et al., 1989; Ho et al., 1989).

DETDESC:

DETD(73)

Testing . . . (Bodner et al., 1988; Ingraham et al., 1988) with DCoH did not enhance the amount of GHF-1-dependent CAT activity. Upon **transfection** of DCoH with the glucocorticoid receptor, enhancement of the ability of the glucocorticoid receptor to activate transcription of the MMTV. . .

US PAT NO: 5,348,864 [IMAGE AVAILABLE] L1: 6 of 18

DRAWING DESC:

DRWD(2)

FIG. . . . Highlighted domains include the leucine-rich domain (shaded box); the acidic region (black box) two proline-rich stretches (open box); two putative **nuclear** **localization** signals (left hatched box) and a cystein-rich region (right hatched box).

DRAWING DESC:

DRWD(8)

FIG. . . . (Katzav, S. et al., supra); (b,c) nude mouse tumors induced by (b) second cycle- and (c) third cycle-transformants derived from **transfection** of NIH3T3 cells with human breast carcinoma DNA and (d) T24 human cells, were digested with Sac I and submitted. . .

DETDESC:

DETD(25)

Expression vectors may be introduced into host cells by various methods known in the art. For example, **transfection** of host cells with expression vectors can be carried out by the calcium phosphate precipitation method. However, other methods for. . .

DETDESC:

DETD(59)

Human . . . plasmids directed the synthesis of the expected vav protein as determined by immunoprecipitation analysis of G418-resistant NIH3T3 cells generated by co-**transfection** of these plasmids with the selectable marker pSV2neo.

DETDESC:

DETD(63)

Transfection of NIH3T3 cells, isolation of transformed cells, selection of G418-resistant colonies, metabolic labeling of cells with [.sup.35 S-]methionine, immunoprecipitation with. . .

DETDESC:

DETD(69)

Other . . . that may represent hinge regions; (iii) a putative protein kinase A phosphorylation site (residues 435 to 440); (iv) two

putative **nuclear** **localization** signals (residues 486 to 493 and 575 to 582); (v) a cysteine-rich sequence which includes two metal binding motifs Cys-X.sub.2. . .

DETDESC:

DETD(71)

Alignment . . . the putative protein kinase A phosphorylation site, the cystein-rich sequence that can fold into zinc finger-like structures and the putative **nuclear** **localization** signals, are also present in a mouse vav gene product (FIG. 2) [see SEQ. ID NO: 1]. The mouse vav. . .

DETDESC:

DETD(82)

Transfection of NIH3T3 cells with pJC11 DNA, an expression plasmid carrying a mouse vav proto-oncogene, did not revealed significant levels of. . . that lacks 65 of the 67 amino-terminal residues absent in the human vav oncogene product (Katzav, S. et al., supra). **Transfection** of NIH3T3 cells with pJC12 DNA resulted in the appearance of about 3,000 foci of transformed cells per microgram of. . .

DETDESC:

DETD(83)

The . . . codon, translation from pJC7 DNA is likely to start in the second in-frame ATG, the initiator codon used by pJC12. **Transfection** of NIH3T3 cells with pJC25 DNA resulted in the appearance of about 40,000 foci of transformed cells per microgram of. . .

US PAT NO: 5,342,774 [IMAGE AVAILABLE] L1: 7 of 18

DETDESC:

DETD(3)

In order to identify and isolate the gene coding for antigen PS15A, gene **transfection** was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line. . .

DETDESC:

DETD(6)

When . . . of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the **transfection** experiments.

DETDESC:

DETD(10)

Previous . . . 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library **transfection** to recover genes coding for tum.sup.- antigens.

DETDESC:

DETD(11)

The entire plasmid and genomic DNA of P1.HTR was prepared, following Wolfel et al., Immunogenetics 26: 178-187 (1987). The **transfection** procedure followed Corsaro et al., Somatic Cell Molec. Genet 7:603-616 (1981), with some modification. Briefly, 60 .mu.g of cellular DNA. . . flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after **transfection**, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350. . .

DETDESC:

DETD(14)

Eight days after **transfection** as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These. . .

DETDESC:

DETD(25)

Using . . . supra, (60 ug) and 4 ug of pHMR272, described supra, groups of 5.times.10.sup.6 PO.HTR cells were used as transfectant hosts. **Transfection** was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group. . .

DETDESC:

DETD(27)

As . . . 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, **transfection** and amplification of the cosmids followed example 5, again using PO.HTR.

DETDESC:

DETD(28)

High frequencies of **transfection** were observed, as described in Table 1, which follows:

DETDESC:

DETD(34)

This . . . was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after **transfection**. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e.,. . .

DETDESC:

DETD(49)

Analysis . . . has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84:5306-5310 (1987), in a murine heterodomain protein having **nuclear** **localization**.

DETDESC:

DETD(63)

The . . . shows phenotype H-2.sup.k. The cell lines were transfected with genes expressing one of the K.sup.d, D.sup.d, and L.sup.d antigen. Following **transfection** with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized. . .

DETDESC:

DETD(70)

In . . . conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high **transfection**

frequency, i.e., it must be a "good" recipient.

DETDESC:

DETD(74)

Following . . . Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after **transfection**, the cells were harvested and seeded at 4.times.10.sup.6 cells per 80 cm.sup.2 flask in melanoma culture medium supplemented with 2. . .

DETDESC:

DETD(76)

Thirteen days after **transfection**, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated. . .

DETDESC:

DETD(83)

Cells . . . production as discussed in Example 17, supra. A total of 100 groups of E.sup.- cells (4.times.10.sup.6 cells/group) were tested following **transfection**, and 7.times.10.sup.4 independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected. . .

DETDESC:

DETD(89)

The . . . preparation of a cosmid library. This library of nearly 50,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid **transfection** protocols described supra. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion. . . fragments were cloned into vector pTZ 18, and then into MEL2.2. Again, TNF production was the measure by which successful **transfection** was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12. . .

DETDESC:

DETD(134)

Cells . . . such as interleukins (e.g., IL-2 or IL-4), or major histocombatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene **transfection** is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of. . .

DETDESC:

DETD(135)

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by. . . particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA, additional **transfection** is not necessary. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLAmolecule. It is to be understood, of course, that **transfection** with one additional sequence does not preclude further **transfection** with other sequences.

DETDESC:

DETD(137)

Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for. . .

DETDESC:

DETD(138)

The . . . where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-**transfection** is a well known technique, and the artisan in this field is expected to have this technology available for utilization.

US PAT NO: 5,310,662 [IMAGE AVAILABLE] L1: 8 of 18

SUMMARY:

BSUM(10)

It . . . sequences have identified certain functional domains within the molecule that are thought responsible respectively for DNA binding, hormone binding and **nuclear** **localization**. See Evans, et al.,

Science 240, 889 (1988) for a review of this subject matter. In the case of the. . .

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

DETDESC:

DETD(47)

DNA binding was measured as described previously (Hollenberg et al., supra). Mutant receptor, obtained in a crude COS-1 cell extract after **transfection**, was incubated with a mixture of radiolabeled DNA fragments, one of which contained GREs. Receptor-DNA complexes were immunoprecipitated with receptor-specific. . .

DETDESC:

DETD(48)

Transfection and Luciferase Assays

DETDESC:

DETD(49)

Transfection of CV-1 and COS-1 cells was as described previously (Giguere et al., and Hollenberg et al., supra) using 5 micrograms. . .

DETDESC:

DETD(50)

Cell Culture and **Transfection**

DETDESC:

DETD(51)

Conditions for growth and **transfection** of CV-1 (African green monkey kidney) cells were as previously described (Giguere et al., Cell 46, 645 (1986)), except that. . . Typically, 5 .mu.g reporter and 1 .mu.g expression vector were cotransfected, along with 2.5 .mu.g RSV-.beta.gal as a control for **transfection** efficiency. Acetylated and non-acetylated forms of [.sup.14]chloramphenicol were separated by thin layer chromatography, excised, and quantitated by liquid scintillation. . .

US PAT NO: 5,302,519 [IMAGE AVAILABLE] L1: 9 of 18

SUMMARY:

BSUM(4)

The . . . dimerization domains. The bHLH family includes over 60 proteins in vertebrates, yeast, plants, and insects; many, if not all, exhibit **nuclear** **localization**, are sequence-specific DNA-binding proteins, and function as transcriptional regulators(6). The region of sequence similarity shared to Myc and other proteins. . .

DETDESC:

DETD(132)

Myc . . . Mad-1 to these proteins suggests that it should be localized to the nucleus as well. There is a potential bipartite **nuclear** **localization** (Dingwall and Laskey, (1991) signal in Mad-1 found between amino acids 20 and 50. Myc and Max are in vivo. . .

DETDESC:

DETD(166)

In transient **transfection** assays, Myc activated transcription of a heterologous reporter gene containing the CACGTG binding motif in its promoter while Max repressed. . .

US PAT NO: 5,262,300 [IMAGE AVAILABLE] L1: 10 of 18

SUMMARY:

BSUM(9)

It . . . sequences have identified certain functional domains within the molecule that are thought responsible respectively for DNA binding, hormone binding and **nuclear** **localization**. See Evans, et al., Science 240, 889 (1988) for a review of this subject matter. In the case of the. . .

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

DETDESC:

DETD(48)

DNA binding was measured as described previously (Hollenberg et al., supra). Mutant receptor, obtained in a crude COS-1 cell extract after **transfection**, was incubated with a mixture of radiolabeled DNA fragments, one of which contained GREs, Receptor-DNA complexes were immunoprecipitated with receptor-specific. . .

DETDESC:

DETD(49)

Transfection and Luciferase Assays

DETDESC:

DETD(50)

Transfection of CV-1 and COS-1 cells was as described previously (Giguere et al., and Hollenberg et al., supra) using 5 micrograms. . .

DETDESC:

```
=> s nuclear localization (p) transfection
         49871 NUCLEAR
          5658 LOCALIZATION
```

39 NUCLEAR LOCALIZATION
(NUCLEAR(W)LOCALIZATION)
1723 TRANSFECTION
L2 4 NUCLEAR LOCALIZATION (P) TRANSFECTION
=> d 1-4 kwic

US PAT NO: 5,468,624 [IMAGE AVAILABLE] L2: 1 of 4

DETDESC:

DETD(46)

The . . . 21 missense amino acids and stops. It has some sequence important for binding GREs but lacks signals for transcriptional activation, **nuclear** **localization**, steroid binding, and most sites for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of **transfection**. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . .

US PAT NO: 5,310,662 [IMAGE AVAILABLE] L2: 2 of 4

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

US PAT NO: 5,262,300 [IMAGE AVAILABLE] L2: 3 of 4

DETDESC:

DETD(25)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to

activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.
. .

US PAT NO: 5,217,867 [IMAGE AVAILABLE] L2: 4 of 4

DETDESC:

DETD(24)

The CAT activity measured in the transcription assay is the sum of multiple individual functions including **nuclear** **localization**, DNA binding, dimerization and perhaps the allosteric events and protein-protein interactions that ultimately result in activation. If more than one. . . retain their ability to bind DNA but fail to activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific.
. .

```
=> s nuclear localization (p) liposome
         49871 NUCLEAR
          5658 LOCALIZATION
            39 NUCLEAR LOCALIZATION
                  (NUCLEAR(W)LOCALIZATION)
          1746 LIPOSOME
L3           0 NUCLEAR LOCALIZATION (P) LIPOSOME
=> s nuclear localization (p) cationic lipid
         49871 NUCLEAR
          5658 LOCALIZATION
            39 NUCLEAR LOCALIZATION
                  (NUCLEAR(W)LOCALIZATION)
         41201 CATIONIC
         10143 LIPID
            19 CATIONIC LIPID
                  (CATIONIC(W)LIPID)
L4           0 NUCLEAR LOCALIZATION (P) CATIONIC LIPID
=> s poly l lysine and transfection
         83059 POLY
        452248 L
SEARCH ENDED BY USER
=> s poly l lysine (p) transfection
         83059 POLY
        452248 L
         14346 LYSINE
           484 POLY L LYSINE
                  (POLY(W)L(W)LYSINE)
```

1723 TRANSFECTION
L5 1 POLY L LYSINE (P) TRANSFECTION
=> d kwic

US PAT NO: 5,480,981 [IMAGE AVAILABLE] L5: 1 of 1

SUMMARY:

BSUM(101)

Antisense . . . in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as **poly**-(**L**-**lysine**). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense. . . into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO.sub.4 -mediated DNA **transfection**, electropotation, or other gene transfer vectors such as Epstein-Barr virus. Antisense or sense oligonucleotides are preferably introduced into a cell. . .
=> s poly l lysine (p) liposome
83059 POLY
452248 L

14346 LYSINE
484 POLY L LYSINE
(POLY(W)L(W)LYSINE)
1746 LIPOSOME
L6 5 POLY L LYSINE (P) LIPOSOME
=> d 1-5 kwic

US PAT NO: 5,508,184 [IMAGE AVAILABLE] L6: 1 of 5

SUMMARY:

BSUM(27)

Therefore, . . . electroporation, possibly in conjunction with other processes for gene transfer used in microbiological research such as, for example, poly-L-ornithine or **poly**-**L**-**lysine** treatment, **liposome** fusion, DNA-protein complexing, charge modification on the protoplast membrane, fusion with microbial protoplasts or calcium phosphate correcipitation and, especially, polyethylene. . .

US PAT NO: 5,453,367 [IMAGE AVAILABLE] L6: 2 of 5

DETDESC:

DETD(42)

The . . . this step with techniques which are employed in microbiological research for gene transfer, for example by treatment with poly-L-ornithine or **poly**-**L**-**lysine**, **liposome** fusion, DNA protein complexing, altering the charge at the protoplast membrane, fusion with microbial protoplasts, or calcium phosphate co-precipitation and,. . .

US PAT NO: 5,231,019 [IMAGE AVAILABLE] L6: 3 of 5

DETDESC:

DETD(41)

The . . . this step with techniques which are employed in microbiological research for gene transfer, for example by treatment with poly-L-ornithine or **poly**-**L**-**lysine**, **liposome** fusion, DNA protein complexing, altering the charge at the protoplast membrane, fusion with microbial protoplasts, or calcium phosphate co-precipitation and,. . .

US PAT NO: 4,921,757 [IMAGE AVAILABLE] L6: 4 of 5

DETDESC:

DETD(20)

The . . . entrapped within the liposomes or packaged within the surrounding matrix. To prevent diffusion or release of the enzyme from the **liposome** or matrix, the molecular weight of the enzyme may be increased by any of a number of methods, thereby trapping. . . of substrate to protect the active site. Also, the enzyme could be covalently linked to a soluble polymer such as **poly**-**L**-**lysine**. The phospholipase is selected for ability to cleave one or more of the phospholipids making up the **liposome**, and non-toxicity to the organism or surrounding cells if for in vivo use.

US PAT NO: 4,900,556 [IMAGE AVAILABLE] L6: 5 of 5

DETDESC:

DETD(20)

The . . . entrapped within the liposomes or packaged within the surrounding microcapsule. To prevent diffusion or release of the enzyme from the **liposome** or microcapsule, the enzyme molecular weight may be

increased by a number of methods, causing the enzyme to be trapped. . . of substrate to protect the active site. Also, the enzyme could be covalently linked to a soluble polymer such as **poly**-**L**-**lysine**. The phospholipase is selected for ability to cleave one or more of the phospholipids making up the **liposome**, and non-toxicity to the organism or surrounding cells if for in vivo use.

```
=> s poly l lysine (p) cationic lipid
         83059 POLY
        452248 L

         14346 LYSINE
           484 POLY L LYSINE
                 (POLY(W)L(W)LYSINE)
         41201 CATIONIC
         10143 LIPID
            19 CATIONIC LIPID
                 (CATIONIC(W)LIPID)
L7            0 POLY L LYSINE (P) CATIONIC LIPID
=> s dna binding (p) transfection
         14427 DNA
         81199 BINDING
           501 DNA BINDING
                 (DNA(W)BINDING)
          1723 TRANSFECTION
L8           26 DNA BINDING (P) TRANSFECTION
=> s dna binding domain (p) transfection
         14427 DNA
         81199 BINDING
         21607 DOMAIN
            76 DNA BINDING DOMAIN
                 (DNA(W)BINDING(W)DOMAIN)
          1723 TRANSFECTION
L9           10 DNA BINDING DOMAIN (P) TRANSFECTION
=> d 1-10 kwic

US PAT NO:     5,468,624 [IMAGE AVAILABLE]            L9: 1 of 10

DRAWING DESC:

DRWD(2)

 FIG. 1 shows the results of **transfection** of ICR 27 cells with GR constructs. ICR 27 cells were transfected with 4 different GR constructs, namely holo GR, . . . for cell kill in the absence (-) or presence (+) of 10.sup.-6 M dex for up to 96 hours after **transfection**. The numbers above the boxes correspond to the amino acid position in the protein sequence of the steroid receptor. The. . . the left diagonal hatches,
```

the diamonds, the right diagonal hatches, and the chevrons correspond to the tau 1 domain, the **DNA** **binding** **domain**, the tau 2 domain, and the steroid binding domain, respectively. The percentage reduction in viable cell number, both in the absence (-) or presence (+) of dex, following **transfection** of ICR 27 cells with these steroid receptor constructs is indicated. Superscript "a" indicates results for cell kill that are. . .

DETDESC:

DETD(31)

Transfection of holo glucocorticoid receptor into glucocorticoid-resistant ICR 27 cells could restore cell lysis on addition of 10.sup.-6 M dexamethasone (27,28,30). Since these were transient **transfection** assays, the extent of lysis was not 100%, but averaged 26.+-.4% in 23 assays, each done in triplicate. The holoreceptor. . . dispensable for the cell lysis function. In fact, the present inventors have shown that construct .DELTA.9-385/532* which encodes the central **DNA** **binding** **domain** and sequences flanking it on either side could effectively lyse cells in a constitutive manner (27,28). However no previous tests had been done proving that neither the carboxyl-terminal end of the **DNA** **binding** **domain** nor the amino acids 1-8 and 387-397 could be eliminated with full retention of the lethal function. In order to further delineate the minimal sequence encoding this function, the present inventors deleted progressively from either end towards the central **DNA** **binding** **domain**.

DETDESC:

DETD(33)

A . . . construction is described earlier, was used to transfect ICR 27 cells as shown in FIG. 1. Within 6-24 hours of **transfection** and in the absence of dexamethasone 28% of the cells were lysed--an extent comparable to that evoked by the holoreceptor and steroid 48-96 hours after **transfection**. Thus, a sequence which spans less than 100 amino acids is responsible for the constitutive lethality of the receptor. Although. . . with partial deletions of the amino terminal domain of the GR always included parts of that region and the entire **DNA** **binding** **domain**. That a sequence containing a **DNA** **binding** **domain** mutated in its 3' portion, and only the 22 proximal amino acids at the amino terminal end would be fully. . .

DETDESC:

DETD(34)

The following fragments or mutations of the GR gene were inactive for cell lysis upon **transfection**: .DELTA.420-451 (deletion of the first zinc finger); .DELTA.450-487 (deletion of the second zinc finger); .DELTA.428-490 (deletion of the entire **DNA** **binding** **domain**); I 422 (insertion of three amino acids between the first two cysteines in the first zinc finger); and GTG3A (chimeric GR having modified thyroid hormone receptor **DNA** **binding** **domain** which cannot recognize glucocorticoid response elements). Four point mutants having glycine substitutions in key amino acid positions in the zinc finger structure of the **DNA** **binding** **domain** were selected to test their importance in the cell kill process. The constructs transfected were G424, G442, G455 and G463--a. . .

DETDESC:

DETD(46)

The modified lethal fragment of the receptor, 398-465*, starts 23 amino acids upstream from the **DNA** **binding** **domain**, goes through the first zinc finger, the linker region which follows it and through amino acid position 46. in the. . . for protein:protein interactions. It is constitutively active and can kill cells in which it is expressed within 6-24 hours of **transfection**. The construct is as effective in effecting cell lysis as is the holoreceptor in the presence of steroid. Several of. . . zinc finger structure which binds the GREs in regulated genes. The mutants which had single amino acid substitutions in the **DNA** **binding** **domain** were functionally inactive for cell kill, stressing the importance of an intact **DNA** **binding** **domain** (at least halfway through the second zinc finger).

US PAT NO: [illegible]265 [IMAGE AVAILABLE] L9: 2 of 10

DETDESC:

DETD(4)

Thus, . . . cells are grown in a medium during the first day of the assay detailed below as the "Cationic Liposome Mediated **Transfection** Assay". In the **transfection** procedure, which is performed during the second day of the **transfection** assay, the DNA plasmid coding for the CAT enzyme is also added to each cell culture, in addition to the. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl et al. article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc.

receptor, only then is the receptor bound through its **DNA**-**binding** **domain** to the estrogen response element of the estrogen-response-element-chloramphenicol-acetyl transferase-construct (ERE-CAT). In other words, through multiple interactions CAT enzyme is manufactured. . .

US PAT NO: 5,[illegible]6,150 [IMAGE AVAILABLE] L9: 3 of 10

DETDESC:

DETD(117)

Construction . . . and gel-purified. This fragment was then substituted into the vector pRRSfokIR to replace the DNA segment coding for the FokI **[illegible]-**binding** **domain** and, hence, form the Ubx-F.sub.N hybrid gene (FIG. 17B). After **transfection** of competent RR1 cells with the ligation mix, several clones were identified by restriction analysis and their DNA sequences were. . .

US PAT NO: 5,[illegible]0,586 [IMAGE AVAILABLE] L9: 4 of 10

DETDESC:

DETD(4)

Thus, . . . cells are grown in a medium during the first day of the assay detailed below as the "Cationic Liposome Mediated **Transfection** Assay". In the **transfection** procedure, which is performed during the second day of the **transfection** assay, the DNA plasmid coding for the CAT enzyme is also added to each cell culture, in addition to the. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl. et al. article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta.. . . receptor, only then is the receptor bound through its **DNA**-**binding** **domain** to the estrogen response element of the estrogen-response-element-chloramphenicol-acetyl transferase-construct (ERE-CAT) capable of initiating transcription of messenger RNA for the CAT. .

US PAT NO: [illegible],662 [IMAGE AVAILABLE] L9: 5 of 10

DETDESC:

DETD(25)

The . . . allosteric events and protein-protein interactions that

ultimately resul[illegible] activation. If more than one essential function is encoded by the [illegible]NA** **binding** **domain**, some of the non-functional point mutants ma[illegible]still retain their ability to bind DNA but fail to activate. To ex[illegible]re this possibility, each mutant protein was produced by **transfecti[illegible] of the corresponding expression vector into COS-1 cells and assay[illegible] for its ability, in crude extracts, to form a specific. . .

US PAT NO: [illegible].74,077 [IMAGE AVAILABLE] L9: 6 of 10

DETDESC:

DETD(109)

FIG. . . . [illegible]nsferred to the expression vector pRS (see Giguere, eial., (1986), a[illegible] the Not1/Xhol restriction fragment of pRShGR.sub.NX containing the [illegible]GR **DNA**-**binding** **domain** was introduced into pRShRnx between the Not1 and Xhol sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were intro[illegible]ced into CV-1 cells by calcium phosphate coprecipitation [illegible]ee. . .

US PAT NO: [illegible],762,300 [IMAGE AVAILABLE] L9: 7 of 10

DETDESC:

DETD(25)

The . . . a[illegible]losteric events and protein-protein interactions that ultimately resu[illegible]t in activation. If more than one essential function is encoded by the **DNA** **binding** **domain**, some of the non-functional point mutants may still retain their ability to bind DNA but fail to activate. To exp[illegible]ore this possibility, each mutant protein was produced by **transfecti[illegible] of the corresponding expression vector into COS-1 cells and assay[illegible] for its ability, in crude extracts, to form a specific. . .

US PAT NO: [illegible],117,867 [IMAGE AVAILABLE] L9: 8 of 10

DETDESC:

DETD(24)

The . . . a[illegible]losteric events and protein-protein interactions that ultimately resu[illegible]t in activation. If more than one essential function is encoded by the [illegible]NA** **binding** **domain**, some of the non-functional point mutants m[illegible] still retain their ability to bind DNA but fail to

activate. To explore this possibility, each mutant protein was produced by **transfection** of the corresponding expression vector into COS-1 cells and assayed for its ability, in crude extracts, to form a specific. . .

US PAT NO: 5,171,671 [IMAGE AVAILABLE] L9: 9 of 10

DETDESC:

DETD(109)

FIG. . . . to the expression vector pRS (see Giguere, et al., (1986), and the Not1/Xho1 restriction fragment of pRShGR.sub.NX containing the hGR **DNA**-**binding** **domain** was introduced into pRShRnx between the Not 1 and Xho sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were introduced into CV-1 cells by calcium phosphate coprecipitation (see. . .

US PAT NO: 4,981,784 [IMAGE AVAILABLE] L9: 10 of 10

DETDESC:

DETD(114)

FIG. . . . to the expression vector pRS (see Giguere, et al., (1986), and the Notl/Xhol restriction fragment of pRShGR.sub.NX containing the hGR **DNA**-**binding** **domain** was introduced into pRShRnx between the Notl and Xhol sites to create pRShRGR. B, Cell **transfection** and CAT assay. The recombinant DNA constructs (5 .mu.g each) were introduced into CV-1 cells by calcium phosphate coprecipitation (see. . .

=> s dna binding domain (p) liposome

```
         14427 DNA
         81199 BINDING
         21607 DOMAIN
            76 DNA BINDING DOMAIN
                  DNA(W)BINDING(W)DOMAIN)
          1746 LIPOSOME
L10          2 DNA BINDING DOMAIN (P) LIPOSOME
```

=> d 1-2 kwic

US PAT NO: 5,465,265 [IMAGE AVAILABLE] L10: 1 of 2

DETDESC:

DETD(4)

Thus, . . . that HeLa cells are grown in a medium during the first

day of the assay detailed below as the "Cationic **Liposome** Mediated Transfection Assay". In the transfection procedure, which is performed during the second day of the transfection assay, the DNA. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl et al. article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc. receptor, only then is the receptor bound through its **DNA**-**binding** **domain** to the estrogen response element of the estrogen-response-element-chloramphenicol-acetyl transferase-construct (ERE-CAT). In other words, through multiple interactions CAT enzyme is manufactured. . .

US PAT NO: 5,599,586 [IMAGE AVAILABLE] L10: 2 of 2

DETDESC:

DETD(4)

Thus, . . . that HeLa cells are grown in a medium during the first day of the assay detailed below as the "Cationic **Liposome** Mediated Transfection Assay". In the transfection procedure, which is performed during the second day of the transfection assay, the DNA. . . receptors (which were constructed in accordance with the teachings of the M. Pfahl. et al, article) also contain a a **DNA** **binding** **domain**, which is capable of binding to the "estrogen response element" (a DNA fragment) attached to the DNA plasmid coding for. . . to the ligand binding domain of the respective RAR.sub..alpha., RAR.sub..beta. etc. receptor, only then is the receptor bound through its **DNA**-**binding** **domain** to the estrogen response element of the estrogen-response element-chloramphenicol-acetyl transferase-construct (ERE-CAT) capable of initiating transcription of messenger RNA for the CAT. .

```
=> s dna binding domain (p) cationic lipid
         1442 DNA
         8119 BINDING
         2160 DOMAIN
            7 DNA BINDING DOMAIN
                 (DNA W) BINDING (W) DOMAIN)
         4120 CATIONIC
         1014 LIPID
            1 CATIONIC LIPID
                 (CATIONIC (W) LIPID)
L11           1 DNA BINDING DOMAIN (P) CATIONIC LIPID
=> logoff y
U.S. Patent & Trademark Office LOGOFF AT 11:50:29 ON 24 APR 96
```

TI 11q23 translocations split the "AT-hook" cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene.

AU Zeleznik-Le N J; Harden A M; Rowley J D
CS Department of Medicine, University of Chicago, IL 60637..

NC CA42557 (NCI)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Oct 25) 91 (22) 10610-4.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9501

AB Translocations involving chromosome band 11q23, found in acute lymphoid and myeloid leukemias, disrupt the MLL gene. This gene encodes a putative transcription factor with homology to the zinc fingers and other domains of the Drosophila trithorax gene product and to the "AT-hook" motif of high mobility group proteins. To map potential transcriptional activation or repression domains of the MLL ***protein*** , yeast GAL4 ***DNA*** - ***binding*** ***domain*** and MLL hybrid protein-expressing plasmids were cotransfected with chloramphenicol acetyltransferase reporter plasmids in a transient ***transfection*** system. We found that MLL contains a strong activation domain and a repression domain. The former, located telomeric (3') to the breakpoint region, activated transcription 18-fold to > 200-fold, depending on the promoter and cell line used for ***transfection*** . A repression domain that repressed transcription 4-fold was located centromeric (5') to the breakpoint region of MLL. The MLL AT-hook domain protein was expressed in bacteria and was utilized in a gel mobility shift assay to assess DNA-binding activity. The MLL AT-hook domain could bind cruciform DNA, recognizing structure rather than sequence of the target DNA. In translocations involving MLL, loss of an activation domain with retention of a repression domain and a DNA-binding domain on the der(11) chromosome could alter the expression of downstream target genes, suggesting a potential mechanism of action for MLL in leukemia.

L11 ANSWER 6 OF 15 MEDLINE
AN 94286555 MEDLINE
TI Positive and negative transcriptional control by the TAL1 helix-loop-helix protein.

AU Hsu H L; Wadman I; Tsan J T; Baer R
CS Department of Microbiology, University of Texas Southwestern Medical Center, Dallas 75235..

NC CA46593 (NCI)

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Jun 21) 91 (13) 5947-51.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9409
AB Tumor-specific activation of the TAL1 gene is the most common genetic defect associated with T-cell acute lymphoblastic leukemia. The TAL1 gene products possess a basic helix-loop-helix (bHLH) motif, a ***protein*** -dimerization and ***DNA*** - ***binding*** ***domain*** found in several transcription factors. TAL1 polypeptides interact, in vitro and in vivo, with class A bHLH proteins (e.g., E47) to form heterodimers with sequence-specific DNA-binding activity. In this study, we show that TAL1 can regulate the transcription of an artificial reporter gene that contains binding sites for bHLH heterodimers involving TAL1. Transcription of the reporter is strongly induced by E47-E47 homodimers and moderately induced by TAL1-E47 heterodimers. Thus, in a cellular environment that allows formation of E47-E47 homodimers (e.g., in the absence of Id regulatory proteins) TAL1 can repress transcription by recruiting E47 into bHLH complexes with less transcriptional activity (i.e., TAL1-E47 heterodimers). However, in other settings TAL1 can activate transcription because TAL1-E47 heterodimers are more resistant to negative regulation by Id proteins. Hence, TAL1 can potentially regulate transcription in either a positive or negative fashion.

L11 ANSWER 7 OF 15 MEDLINE
AN 94067166 MEDLINE
TI Mechanism of glucocorticoid induction of the rat plasminogen activator inhibitor-1 gene in HTC rat hepatoma cells: identification of cis-acting regulatory elements.
AU Bruzdzinski C J; Johnson M R; Goble C A; Winograd S S; Gelehrter T D
CS Department of Human Genetics, University of Michigan Medical School, Ann Arbor 48109-0618..
NC CA-22729 (NCI)
AM-07245 (NIADDK)
T32-GM-07544 (NIGMS)
SO MOLECULAR ENDOCRINOLOGY, (1993 Sep) 7 (9) 1169-77.
Journal code: NGZ. ISSN: 0888-8809.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9403

AB Type 1 plasminogen activator inhibitor (PAI-1) is the major physiological inhibitor of plasminogen activation, inhibiting both tissue- and urokinase-type plasminogen activators. In HTC rat hepatoma cells, glucocorticoids increase PAI-1 activity, antigen and mRNA accumulation 3- to 5-fold; this increase is due solely to an increase in the rate of PAI-1 gene transcription. We have identified the cis-acting sequences in the 5'-flanking sequence of the HTC PAI-1 gene that mediate this induction. Analysis of a series of hybrid genes containing various portions of the PAI-1 5'-flanking region fused to the chloramphenicol acetyltransferase reporter gene transfected into HTC cells localized the region involved in the transcriptional regulation by glucocorticoids to between -1237 and -764. Electrophoretic mobility shift assays and DNase-I protection assays showed that a glucocorticoid response element (GRE) 15-mer located at -1212 bound the glucocorticoid receptor ***DNA*** - ***binding*** ***domain*** ***protein*** in a concentration-dependent manner. Mutations created within this GRE eliminated its ability both to confer a glucocorticoid response and to bind the glucocorticoid receptor. When placed upstream of a heterologous promoter in either orientation, this GRE conferred glucocorticoid inducibility. We, therefore, conclude that the sole cis-acting sequence required for the glucocorticoid response of the PAI-1 gene in rat HTC hepatoma cells is the GRE at -1212.

L11 ANSWER 8 OF 15 MEDLINE
AN 93181242 MEDLINE
TI The DNA binding domain of the varicella-zoster virus gene 62 protein interacts with multiple sequences which are similar to the binding site of the related protein of herpes simplex virus type 1.
AU Tyler J K; Everett R D
CS MRC Virology Unit, Glasgow, UK..
SO NUCLEIC ACIDS RESEARCH, (1993 Feb 11) 21 (3) 513-22.
Journal code: O8L. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9306
AB Varicella-zoster virus gene 62 encodes a protein with predicted Mr of 140,000D (VZV 140k) that shares extensive predicted amino acid sequence homology with the major immediate early (IE) transcriptional regulator protein of herpes simplex virus type 1 (HSV-1) Vmw175. The integrity of highly conserved region 2 is essential for the DNA binding and transcriptional regulatory functions of Vmw175. Similarly, an insertion mutation in region 2 (codons 468-641) of 140k eliminates the transcriptional repression and activation functions of this protein. We have expressed a

fragment of 140k which encompasses region 2 as a non-fusion polypeptide in bacteria. This 140k ***DNA*** ***binding*** ***domain*** ***peptide*** (codons 417-646) binds to numerous DNA sequences throughout the VZV gene 62 promoter region. It induces multiple regions of protection from DNase I digestion, flanked by sites of DNase I hypersensitivity. Several of the sites recognized can be considered to be divergent forms of the consensus sequence which is recognized by Vmw175. However, by use of a panel of mutagenized probe fragments, we found that the 140k DNA binding domain was less sequence-specific than Vmw175 in its interactions with DNA. Consistent with this, the homologous Vmw175 DNA binding domain, and also intact Vmw175, recognize the gene 62 binding sites much less efficiently than the 140k DNA binding domain. Also in contrast to the situation with Vmw175, the 140k DNA binding domain failed to induce DNA bending when occupying the binding sites in its own promoter. Deletion analysis has mapped the minimal DNA binding domain of the VZV 140k protein, as measured in gel retardation analysis, to lie within residues 472 to 633. The differences in binding characteristics of the DNA binding domains of the homologous VZV 140k and HSV-1 Vmw175 IE proteins may account for the subtle differences in their regulatory activities in ***transfection*** assays and during virus growth in tissue culture.

L11 ANSWER 9 OF 15 MEDLINE
AN 92380993 MEDLINE
TI Localization of O-GlcNAc modification on the serum response transcription factor.
AU Reason A J; Morris H R; Panico M; Marais R; Treisman R H; Haltiwanger R S; Hart G W; Kelly W G; Dell A
CS Department of Biochemistry, Imperial College of Science, Technology, and Medicine, London, United Kingdom..
NC HD 13563 (NICHD)
CA 42486 (NCI)
HD 13563 (NICHD)
+
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 25) 267 (24) 16911-21. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9212
AB A unique form of nucleoplasmic and cytoplasmic protein glycosylation, O-linked GlcNAc, has previously been detected, using Gal transferase labeling techniques, on a myriad of proteins (for review see Hart, G. W., Haltiwanger, R. S., Holt, G. D., and Kelly, W. G. (1989a) Annu. Rev. Biochem. 58, 841-874), including many RNA

polymerase II transcription factors (Jackson, S. P., and Tjian, R. (1988) Cell 55, 125-133). However, virtually nothing is known about the degree of glycosylation at individual sites, or, indeed, the actual sites of attachment of O-GlcNAc on transcription factors. In this paper we provide rigorous evidence for the occurrence and locations of O-GlcNAc on the c-fos transcription factor, serum response factor (SRF), expressed in an insect cell line. Fast atom bombardment mass spectrometry (FAB-MS) of proteolytic digests of SRF provides evidence for the presence of a single substoichiometric O-GlcNAc residue on each of four peptides isolated after sequential cyanogen bromide, tryptic, and proline specific enzyme digestion: these peptides are 306VSASVSP312, 274GTTSTIQTAP283, 313SAVSSADGTVLK324, and 374DSSTDLTQTSSSGTVTLP391. Using an array of techniques, including manual Edman degradation, aminopeptidase, and elastase digestion, together with FAB-MS, the major sites of O-GlcNAc attachment were shown to be serine residues within short tandem repeat regions. The highest level of glycosylation was found on the SSS tandem repeat of peptide (374-391) which is situated within the transcriptional activation domain of SRF. The other glycosylation sites observed in SRF are located in the region of the ***protein*** between the ***DNA*** ***binding*** ***domain*** and the transcriptional activation domain. Glycosylation of peptides (274-283) and (313-324) was found to occur on the serine in the TTST tandem repeat and on serine 316 in the SS repeat, respectively. The lowest level of glycosylation was recovered in peptide (306-312) which lacks tandem repeats. All the glycosylation sites identified in SRF are situated in a relatively short region of the primary sequence close to or within the transcriptional activation domain which is distant from the major sites of phosphorylation catalyzed by casein kinase II.

L11 ANSWER 10 OF 15 MEDLINE
AN 92372658 MEDLINE
TI ***DNA*** - ***binding*** ***domain*** of RCC1 ***protein*** is not essential for coupling mitosis with DNA replication.
AU Seino H; Hisamoto N; Uzawa S; Sekiguchi T; Nishimoto T
CS Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan..
SO JOURNAL OF CELL SCIENCE, (1992 Jul) 102 (Pt 3) 393-400.
Journal code: HNK. ISSN: 0021-9533.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9211
AB The RCC1 protein that is required for coupling mitosis with the S

phase has a DNA-binding domain in the N-terminal region outside the repeat. We found that RCC1 protein without any DNA-binding activity complemented the tsBN2 mutation with the same efficiency as that of intact RCC1 protein. In ts+ transformants of tsBN2 cells transfected with the RCC1 cDNA lacking the DNA-binding domain, an endogenous RCC1 disappeared at 39.5 degrees C, and the deleted RCC1 protein encoded by the transfected cDNA was found in the cytoplasm, but a significant amount of it was also found in the nuclei. This deleted RCC1 protein was eluted from the nuclei with the same concentration of NaCl and DNase I as was used for the intact RCC1 protein in BHK21 cells. Furthermore, the deleted RCC1 protein co-migrated with the nucleosome fraction on sucrose density gradient analysis. These results indicate that the RCC1 protein binds chromatin with the aid of other unknown protein(s). Thus, the ***DNA*** - ***binding*** ***domain*** of RCC1 ***protein*** is not essential for coupling between the S and M phases, but was shown instead to function as a nuclear translocation signal.

L11 ANSWER 11 OF 15 MEDLINE
AN 90356384 MEDLINE
TI The lack of transcriptional activation of the v-erbA oncogene is in part due to a mutation present in the ***DNA*** ***binding*** ***domain*** of the ***protein*** .
AU de Verneuil H; Metzger D
CS Laboratoire de Genetique Moleculaire des Eucaryotes du CNRS, Unite 184 de Biologie Moleculaire, Faculte de Medecine, Strasbourg, France..
SO NUCLEIC ACIDS RESEARCH, (1990 Aug 11) 18 (15) 4489-97.
Journal code: O8L. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9011
AB Using a transient co- ***transfection*** system we have demonstrated that response elements for estrogen (ER), thyroid hormone (TR) and retinoic acid receptors (RAR) are closely related. Thyroid hormone-induced activation of transcription was observed in CV1 cells and not in HeLa cells, suggesting the existence of cell-specific transcription factors necessary for the response. By contrast to its cellular counterpart (c-erbA/cTR alpha) the oncogene protein gag v-erbA is unable to activate gene transcription from different response elements derived from the rat growth hormone (rGH) gene promoter. A chimeric construct consisting of the ER in which the DNA binding domain has been replaced by that of cTR alpha was able to stimulate the reporter gene. In contrast, a construct in which ER DNA binding domain has been replaced by that of gag v-erbA

did not activate gene transcription. These results lead us to the conclusion that the mutated DNA binding domain of v-erbA is in part responsible for the lack of transcriptional activation and in repression of gene expression. This is due in large part to the Gly73----Ser mutation which corresponds to the position of one of the three discriminating amino acids that are thought to interact with a specific base of the response element.

L11 ANSWER 12 OF 15 MEDLINE
AN 90128258 MEDLINE
TI Activation of transcription by v-myb: evidence for two different mechanisms.
AU Klempnauer K H; Arnold H; Biedenkapp H
CS Zentrum fur Molekulare Biologie, Universita Heidelberg, FRG..
SO GENES AND DEVELOPMENT, (1989 Oct) 3 (10) 1582-9.
Journal code: FN3. ISSN: 0890-9369.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9005
AB The retroviral oncogene v-myb encodes a nuclear, sequence-specific DNA-binding protein. To investigate the possibility that v-myb encodes a transcriptional regulator, we used a transient cotransfection assay to explore the potential of v-myb to influence the expression of other genes. We found that expression of a chicken lysozyme promoter/CAT gene construct was activated by v-myb in the presence of myb-specific binding sites. Action was not observed with a truncated v-myb ***protein*** lacking its ***DNA*** - ***binding*** ***domain*** . We also observed that expression of a hybrid human HSP70 promoter/CAT gene, lacking myb-specific binding sites, was activated by v-myb. However, in this case, the truncated v-myb protein, which lacked its DNA-binding domain, also activated HSP70/CAT expression, indicating that trans-activation of this gene construct was independent of the sequence-specific DNA-binding activity of the v-myb protein. These observations suggest that v-myb encodes a trans-activator and that activation of gene expression occurs by two different mechanisms, one of which involves specific binding of v-myb protein to the regulated gene.

L11 ANSWER 13 OF 15 MEDLINE
AN 89311606 MEDLINE
TI Functional characterization of a complex ***protein*** - ***DNA*** - ***binding*** ***domain*** located within the human immunodeficiency virus type 1 long terminal repeat leader region.
AU Malim M H; Fenrick R; Ballard D W; Hauber J; Bohnlein E; Cullen B R

CS Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710..
SO JOURNAL OF VIROLOGY, (1989 Aug) 63 (8) 3213-9.
Journal code: KCV. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 8910
AB Transcriptional trans activation of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) by the viral tat trans activator is mediated by an LTR-specific sequence located immediately 3' to the start of transcription initiation. We have used a range of molecular techniques to examine DNA-protein interactions that occur in the vicinity of this cis-acting sequence. Our results demonstrate the existence of a sequence-specific DNA-protein interaction involving the HIV-1 leader DNA and map this binding event to between -2 and +21 base pairs relative to the HIV-1 LTR transcription start site. Evidence suggesting that this interaction involves three distinct protein-DNA contact sites extending along one side of the DNA helix is presented. Mutation of these sites was found to ablate protein-DNA binding yet was observed to have no effect on either the basal or tat trans-activated level of HIV-1 LTR-specific gene expression. We therefore conclude that this DNA-protein interaction has a function distinct from the regulation of HIV-1 LTR-specific gene expression.

L11 ANSWER 14 OF 15 MEDLINE
AN 89261819 MEDLINE
TI Functional domains of the human vitamin D3 receptor regulate osteocalcin gene expression.
AU McDonnell D P; Scott R A; Kerner S A; O'Malley B W; Pike J W
CS Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030..
NC AR-38170
DK-38130
HD-07857
SO MOLECULAR ENDOCRINOLOGY, (1989 Apr) 3 (4) 635-44.
Journal code: NGZ. ISSN: 0888-8809.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 8909
AB The human vitamin D receptor (VDR) has been cloned recently. Two cDNAs comprising the full-length VDR were spliced, cloned into a mammalian expression vector, and transiently expressed in COS-1

cells. The protein product exhibited properties consistent with that observed for receptor in human cells. A series of 5'- and 3'-deletions of the full-length VDR cDNA was prepared and evaluated. Native DNA binding was localized to a peptide fragment (residues 1-114) whose most prominent feature is the cysteine rich region proven to represent the DNA binding domain in other steroid receptors. Steroid binding-competence required synthesis of a peptide that initiated C-terminal to the DNA-binding domain at residue 114 and which contained the remaining 313 residues. To determine the location of elements within the receptor necessary for transcription, an osteocalcin gene promoter-chloramphenicol acetyltransferase reporter gene was cotransfected together with wild type or mutant VDR cDNAs and the latter's effect on chloramphenicol acetyltransferase activity was assessed. Cotransfection of wild type receptor alone resulted in efficient transcription of the reporter plasmid. However, synthesis of a ***peptide*** containing the ***DNA*** ***binding*** ***domain*** as well as 76 residues carboxy terminal to this region exhibited some degree of activity, albeit constitutive. These results suggest that the functional domains of the VDR are similar to that of other steroid receptors and that these domains participate in the transcriptional regulation of the human osteocalcin gene.

L11 ANSWER 15 OF 15 MEDLINE
AN 89072761 MEDLINE
TI Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets.
AU Hughes M R; Malloy P J; Kieback D G; Kesterson R A; Pike J W; Feldman D; O'Malley B W
CS Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030..
SO SCIENCE, (1988 Dec 23) 242 (4886) 1702-5.
Journal code: UJ7. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 8903
AB Hypocalcemic vitamin D-resistant rickets is a human genetic disease resulting from target organ resistance to the action of 1,25-dihydroxyvitamin D3. Two families with affected children homozygous for this autosomal recessive disorder were studied for abnormalities in the intracellular vitamin D receptor (VDR) and its gene. Although the receptor displays normal binding of 1,25-dihydroxyvitamin D3 hormone, VDR from affected family members has a decreased affinity for DNA. Genomic DNA isolated from these families was subjected to oligonucleotide-primed DNA amplification,

and each of the nine exons encoding the receptor protein was sequenced for a genetic mutation. In each family, a different single nucleotide mutation was found in the ***DNA*** ***binding*** ***domain*** of the ***protein*** ; one family near the tip of the first zinc finger (Gly----Asp) and one at the tip of the second zinc finger (Arg----Gly). The mutant residues were created in vitro by oligonucleotide directed point mutagenesis of wild-type VDR complementary DNA and this cDNA was transfected into COS-1 cells. The produced protein is biochemically indistinguishable from the receptor isolated from patients.

```
=> s (protein or peptide) (2a) dna binding domain and liposome

        593672 PROTEIN
        155363 PEPTIDE
        380545 DNA
        357469 BINDING
         42934 DOMAIN
          2161 DNA BINDING DOMAIN
                 (DNA(W)BINDING(W)DOMAIN)
           111 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN
          3760 LIPOSOME
L12          0 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN AND LIPOSOME

=> s (protein or peptide) (2a) dna binding domain and cationic lipid?

        593672 PROTEIN
        155363 PEPTIDE
        380545 DNA
        357469 BINDING
         42934 DOMAIN
          2161 DNA BINDING DOMAIN
                 (DNA(W)BINDING(W)DOMAIN)
           111 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN
          6579 CATIONIC
        141113 LIPID?
            77 CATIONIC LIPID?
                 (CATIONIC(W)LIPID?)
L13          0 (PROTEIN OR PEPTIDE) (2A) DNA BINDING DOMAIN AND CATIONIC
               LIPID?

=> LOGOFF Y

COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY     SESSION
FULL ESTIMATED COST                              27.10      27.25
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STN INTERNATIONAL LOGOFF AT 12:29:36 ON 24 APR 96

Paper 9

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FILE 'HOME' ENTERED AT 11:54:42 ON 24 APR 96

=> file medline

COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY     SESSION
FULL ESTIMATED COST                                       0.15        0.15

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 OR CONCERNS THIS MAY HAVE CAUSED.  USERS SHOULD DISREGARD THOSE PRIOR
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*** THIS FILE MAY NOT BE CURRENT.  PLEASE SEE HELP CURRENCY FOR   ***
*** DETAILS.                                                      ***

=> s nuclear  oc.lization and transfection

        130517 NUCLEAR
         68463 LOCALIZATION
           118 NUCLEAR LOCALIZATION
                 (NUCLEAR(W)LOCALIZATION)
         29982 TRANSFECTION
L1         19  NUCLEAR LOCALIZATION AND TRANSFECTION

=> s nuclear .ocalization signal and transfection

        130582 NUCLEAR
         68463 LOCALIZATION
         79193 SIGNAL
            31 NUCLEAR LOCALIZATION SIGNAL
                 (NUCLEAR(W)LOCALIZATION(W)SIGNAL)
         29982 TRANSFECTION
L2          1  NUCLEAR LOCALIZATION SIGNAL AND TRANSFECTION
```

=> s (peptide or protein) and nuclear localization signal and transfection

```
         1553   PEPTIDE
         5936   PROTEIN
         1305   NUCLEAR
          684   LOCALIZATION
          791   SIGNAL
            3   NUCLEAR LOCALIZATION SIGNAL
                  (NUCLEAR(W)LOCALIZATION(W)SIGNAL)
          299   TRANSFECTION
L3              (PEPTIDE OR PROTEIN) AND NUCLEAR LOCALIZATION SIGNAL AND T
                RANSFECTION
```

=> d 1-10 bib ab

L2 ANSWER 1 OF 64 MEDLINE
AN 96102146 MEDLINE
TI An amino acid sequence motif sufficient for subnuclear localization of an arginine/serine-rich splicing factor.
AU Hedley M L; Amrein H; Maniatis T
CS Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA.
NC GM42231 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (1995 Dec 5) 92 (25) 11524-8.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9603
AB We have identified an amino acid sequence in the Drosophila Transformer (Tra) ***protein*** that is capable of directing a heterologous ***protein*** to nuclear speckles, regions of the nucleus previously shown to contain high concentrations of spliceosomal small nuclear RNAs and splicing factors. This sequence contains a nucleoplasmin-like bipartite ***nuclear*** ***localization*** ***signal*** (NLS) and a repeating arginine/serine (RS) dipeptide sequence adjacent to a short stretch of basic amino acids. Sequence comparisons from a number of other splicing factors that colocalize to nuclear speckles reveal the presence of one or more copies of this motif. We propose a two-step subnuclear localization mechanism for splicing factors. The first step is transport across the nuclear envelope via the nucleoplasmin-like NLS, while the second step is association with

compo[illegible] the speckled dom in via the RS dipeptide sequence.

L[illegible] ANSWER [illegible] 64 MEDLINE
AN 960790[illegible] MEDLINE
TI Effic[illegible] clear localization and immortalizing ability, two functio[illegible] ependent on the ade ovirus type 5 (Ad5) E1A second exon, are nec[illegible] y for co ransforma ion with Ad5 E1B but not with T24ras.
AU Dougla[illegible] L; Quinla[illegible] M P
CS Departm[illegible] of Microbiology and Immunology, University of Tennessee Health [illegible]ence Cente, Memphis 38163, USA.
NC CA-505[illegible] ([illegible])
SO JOURNAL [illegible] IROLOGY, (1995 Dec) 69 (12) 8061-5.
Journal [illegible]: KCV. [illegible]SSN: 0022-[illegible]38X.
CY United [illegible]
DT Journal [illegible]rticle; (JOURNAL ARTICLE)
LA English.
FS Priorit[illegible] Journals; Cancer Journals
EM 9603
AB Expressi[illegible] of adenovirus type [illegible] E1A 12S is sufficient to immortalize primar[illegible] rat kidney cells, but another viral or cellular oncogen[illegible] such as E1B or T24ras, is necessary for complete transfo[illegible]ion. The regions of 12S sufficient for T24ras cotrans[illegible]mation have been well characterized and are located in the first e[illegible]n. The second exon is dispensable for ras cotransformation, althou[illegible] contains a region which appears to modulate the transfo[illegible] phenotype. The same 12S first exon regions important in ras t[illegible]sformation are also necessary for E1B transformation. Analysi[illegible] of an extensive series of second exon deletion and amino acid po[illegible] mutations demonstrated that mutations affecting either the en[illegible]ent nuclear localization and/or the immortalizing ability of the [illegible] ***protein*** also prevented cooperation with E1B. In general[illegible] the entire C-terminal half of 12S, including the ***nu[illegible]*** ***localizat on*** ***signal*** , was necessa[illegible] for effici[illegible]nt cotransformation with E1B. In addition to the di[illegible]nces between T24ras and E1B regarding 12S regions necessa[illegible] for cotransfo mation, the characteristics of E1B-co[illegible]nsformed foci differed from those of T24ras. The E1B foci took [illegible] to appea[illegible] and had a much slower growth rate. No hyper[illegible]rmed fo[illegible] were pro[illegible]uced with E1B cotransfections, and estab[illegible]shed E1A-E1B [illegible]nes exhibited minimal growth in soft agar compa[illegible]d to that [illegible] E1A-T24ras lines.

L[illegible] ANSWER[illegible] OF 64 MEDLINE
AN 96074[illegible] MEDLINE
TI Mutat[illegible]nalysis [illegible] p80 coi[illegible]in indicates a functional interaction betwee[illegible] [illegible]ed bodie[illegible] and the nucleolus.
AU Bohmann[illegible] Ferreira [illegible] Lamond A I

CS European Molecular Biology Laboratory, Heidelberg, Germany..
SO JOURNAL OF CELL BIOLOGY, (1995 Nov) 131 (4) 817-31.
Journal code: HMV. ISSN: 0021-9525.
CY United States
DT Journal Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9602
AB Coiled bodies are conserved subnuclear domains found in both plant and animal cells. They contain a subset of splicing snRNPs and several nucleolar antigens, including Nopp140 and fibrillarin. In addition, autoimmune patient sera have identified a coiled body specific ***protein*** called p80 coilin. In this study we show that p80 coilin is ubiquitously expressed in human tissues. The full-length human p80 coilin ***protein*** correctly localizes in coiled bodies when exogenously expressed in HeLa cells using a transient ***transfection*** assay. Mutational analysis identifies separate domains in the p80 coilin ***protein*** that differentially affect its subnuclear localization. The data show that p80 coilin has a ***nuclear*** ***localization*** ***signal***, but this is not sufficient to target the ***protein*** to coiled bodies. The results indicate that localization of p80 coilin in coiled bodies is not determined by a simple motif analogous to the NLS motifs involved in nuclear import. A specific carboxy-terminal deletion in p80 coilin results in the formation of pseudo-coiled bodies that are unable to recruit splicing snRNPs. This is not a loss of endogenous coiled bodies. A separate class of mutant p80 proteins are shown to localize in fibrillar structures that surround nucleoli. These mutants also lead to loss of endogenous coiled bodies, produce a dramatic disruption of nucleolar architecture and cause a specific segregation of nucleolar antigens. The structural changes in nucleoli is accompanied by the loss of RNA polymerase I activity. These data indicate that p80 coilin plays an important role in subnuclear organization and suggest that there may be a functional interaction between coiled bodies and nucleoli.

L3 ANSWER 4 OF 4 MEDLINE
AN 96069[illegible] MEDLINE
TI Initiation binding [illegible] factor that binds to the transcription initiation site of the histone h5 gene, is a glycosylated member of a family of cell growth regulators [corrected and published erratum appears in Mol Cell Biol 1996 Feb].
AU Gonen-C[illegible] P; Lo A; Ha[illegible] Abel M; Ruiz-Carrillo A
CS Cancer Research Center, Medical School of Laval University, L'Hotel-Dieu de Quebec, Canada
SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Dec) 15 (12) 6670-85.

Journal ... NGY. ... -7306.
CY Unite...
DT Journ... cle; ... CLE)
LA Engli...
FS Prior... Journals
EM 9603
AB ... nding ... [corrected] (IBR) is a chicken erythr... factor ... molecular mass, 70 to 73 kDa) that binds ... sequen... spanning the transcription initiation site of the h... gene ... repressing its transcription. A variety of other ... including transformed erythroid precursors, do not have IBR ... factor referred to as IBF (68 to 70 kDa) that recognizes the sa... sites ... have cloned the IBR cDNA and studied the relat... of IBR ... IBR is a 503-amino-acid-long acidic ***pr... which ... identical to the recently reported human ... alpha-... factor and highly related to the invertebrate transcription factor P3A... and erected wing gene product (EWG). We present ... that IBR and IBF are most likely identical prote... differing in their degree of glycosylation. We have analy... aspects of IBR/F and shown that the facto... ates a... homodimers and that the dimer is the relev... binding ... The evolutionarily conserved N-termi... half of ... R/F ... rs the DNA-binding/dimerization domain (ou... 127 t... 283), one or several casein kinase II sites (37 ... nd a ... ***nuclear*** ***localization*** ***sig... (89 ... which appears to be necessary for nuclear targe... inding ... on revealed that the alternating RCG... Y ... nsens... s high-affinity IBR/F binding sites and t... h direct ... pea... lindrome TGCGCATGCGCA is the optimal site. ... of genes potentially regulated by this family of facto... rily ... genes involved in growth-related metab...

L3 ANSWER ... MED...
AN 960...   MEDL...
TI The t... forms o... ouse ... minal deoxynucleotidyl transferase differ ... the a... lity to add N regions and subcellular local...
AU Bent... Fa... M; Nguyen Q T; Martinez O; Rougeon F; Doyen ...
CS Unit... Genetique ... du Developpement, Paris Cedex 15, France
SO EMBO ... (199... ep 1... (17) 4221-9.
Journal ... EMB... -4189.
CY ENG... ed Kingdom
DT Jour... le; ... CLE)
LA Engl...

FS Prio... ...nals
EM ...601
AB Two ... terminal deoxynucleotidyl transferase ... h code respectively for proteins of ... 9 and 29 ... been previously identified in the mouse thymus ... we show that ... same two transcripts are also present in B ... cells from bone marrow. In addition we demonstrate that the ... ding ... acid insertion found near the carboxy ... al ... significantly alters the function of the enzyme ... , TdTL does not catalyse N region insertion ... the ... on junction of a V(D)J site-specific recombination substrate ... n attempt to explain the lack of N region ... ions we have ... racterized the different parameters which ... ish ... two isoforms of TdT. Examination of trans... ell ... vealed a reduced capacity of TdTL to add nucleotides to the ... DNA, consistent with a lower terminal transferase activity ... more, the half-life of the TdTL ***protein*** in ... se ... s is 2-fold shorter than that of TdTS. Finally ... despite the fact that TdTL has the same ***nuclear*** ***localization*** ... ***signal*** as TdTS, the cellular localization of the ... forms was strikingly different. In contrast ... nuclear ... was found exclusively in the cytoplasm ... stics could contribute to the functional difference ... the two isoforms of TdT. However, the subcellular localization ... TdTL on its own can account for its inability to add N ...

LS ANSWER ...
AN ... 5...
TI ... main of human p53 is dispensable for both transcriptional ... and inhibition of tumor cell growth.
AU Pelle... ... mbridge E J
CS Depa... Micro... and Molecular Genetics, University of Cali... vin...
NC CA ... 0...
CA ... 0... N
SO ONCOGENE ... 95 ... 337-49.
Journal code: ONC ... 9232.
CY ENGLAND ... d ...
DT Journal ... le ... (ARTICLE)
LA English
FS Priority ... als
EM ... 1
AB ... the C-terminal domains of the human p53 ... its growth suppressive and transcriptional ... operties deletion mutants were ... p53 delta 363), 60 (p53 delta 333)

[illegible] acids from the C-terminus of the p53 [illegible] has lost the highly basic tail of the [illegible]-366). p53 delta 333 and p53 delta 306 [illegible] (residues 320-360); p53 delta 306 [illegible] the [illegible] nuclear*** ***localization*** [illegible] residues 316-325). These mutants were [illegible] assayed for [illegible] from two p53 consensus binding sites and [illegible] of two promoter systems in Calu6 lung cancer cells [illegible] over, their ability to inhibit cell growth in [illegible] with a defined p53 status was analysed. [illegible] domain correlated with significant [illegible] from a genomic sequence; (b) [illegible] transcription [illegible] (c) the ability to inhibit colony formation [illegible] not a prerequisite for transactivation [illegible] p53 [illegible] behaved similarly to wt p53 in all the assays [illegible] inducible expression system for p53 [illegible] 363 [illegible] sarcoma cell line known to be growth-sup[illegible] The induction of p53 delta 363 expression [illegible] proliferation albeit to a lesser [illegible] than [illegible] p53 delta 363 could upregulate WAF1/CIP1 [illegible] MDM2 genes. Thus, the basis tail of p53 appears not [illegible] the biological functions of the [illegible] protein.

L[illegible] ANSWER 7 [illegible]

A[illegible] [illegible]9598 [illegible]

T[illegible] Multiple [illegible] of human heat shock transcription factor 1.

AU [illegible] Rur[illegible]

CS [illegible] Department [illegible] Molecular Biology, University of [illegible] School [illegible] Florida 33101, USA..

SO [illegible]CULAR [illegible] (1995 Aug) 15 (8) 4319-30. Journal code [illegible]-7306.

CY [illegible] St[illegible]

DT Journal; [illegible] ARTICLE)

LA [illegible]sh

FS [illegible]ty [illegible]

E[illegible]

A[illegible] [illegible] heat [illegible] human heat shock transcription factor 1 (hHSF1) is [illegible] monomer, acquires DNA-binding ability, is transported [illegible] and becomes transcriptionally competent. [illegible] not [illegible] whether these regulatory changes are [illegible] by [illegible] event or whether they occur independently [illegible] other, providing a multilayered control [illegible] may [illegible] activation of hHSF1. Comparison of [illegible] type [illegible] expressed in Xenopus oocytes and human [illegible] cells [illegible] retention of hHSF1 in the monomeric form

ds or l (LZ1 to LZ3) and a carboxy-terminal
nce el well as on the presence of a titratable
in t cation of hHSF1 appears to induce
indin s to uncover an amino-terminally
d *localization*** ***signal*** . A
ism ntrolling oligomerization regulates
ranscr ce of hHSF1. Components of this
ism w egion, including LZ2 and nearby
nces d that is clearly separated from the
y-ter nscription activation domain(s). We
se th d-back structure that masks the
cript in in the unstressed cell but is opened
modi nHS and/or binding of a factor facilitating
unfol ed cell. Activation of hHSF1 appears to
e at ently regulated structural transitions.

L3 8 O
AN 915
TI lar novel transcription factor that
ols st on.
AU ; Mos ; T
CS de B Severo Ochoa (Consejo Superior de
tigac iversidad Autonoma de Madrid), Spain..
SC JLAR , (1995 Jun) 15 (6) 3164-70.
al co 306.
CY d Stat
DT al; Art ICLE)
LA sh
FS ty Jo
OS K-U
EM
AB elysin metalloproteinases with the widest
rate sp a critical role in tumor invasion and
stasis. reported an element (SPRE) of the
elysin between nucleotides -1221 and -1203
s no nt for the control of stromelysin gene
ssion ation, which induces a nuclear activity
binds ing a concatenated probe with several
s of th en a lambda gt11 cDNA expression
brary fro fibroblasts, we report here the
ular c ding for a novel ***protein***
(of binds to this element and has several
es o ctor, such as a putative leucine
reg ***localization***
gnal* omain with homology to the DNA-binding
s of ce that SPBP is at least a critical
ent o ed SPRE nuclear binding activity is

...esulted [illegible] he ***transfection*** of an
...pression [illegible] ...ansactivates reporter chloramphenicol
...l ...trans... [illegible] ...ntaining either the full-length
...romelysin [illegible] ...le copy of the SPRE cloned upstream of
...e herpes [illegible] ...dine kinase minimal promoter.
Therefore [illegible] ...ed here identify a novel transcription
...tor cri... [illegible] the control of stromelysin expression.

L3 ... 9 OF ... [illegible]
AN ...27 ...70 [illegible]
TI ...tational [illegible] ...la-zoster virus major immediate-early
***protein...
AU ...ux L... [illegible] ...onbroodt S; Merville M P; Rentier B;
...ette J
CS ...Department of M... [illegible] ...niversity of Li`ege, Belgium..
SO ...IC ACI... R... [illegible] Apr 25) 23 (8) 1341-9.
...urnal code... [illegible] ...1048.
CY ...LAND: U... [illegible]
DT J...rnal; A... [illegible] ...TICLE)
LA ...glish
FS ...ority Jou... [illegible] ...nals
EM ...8
AB ...e varicel... [illegible] ...V) open reading frame 62 encodes an
...mediate... [illegible] (IE62) that transactivates
e...pression [illegible] ...moters and autoregulates its own
...pression [illegible] ...ssion assays. In Vero cells, IE62 was
...own to tra... [illegible] ...ession of all putative
...mediate-e...y [illegible] ...(IE) genes of VZV with an
...regulati... [illegible] ...ntracellular concentrations. To define
...function... [illegible] ...ed in the regulatory properties of
...2, a la... [illegible] ...ame insertions and deletions were
...roduced [illegible] ...e copy of the gene encoding IE62.
...udies of [illegible] ...ities of the resultant mutant
...ypeptide [illegible] ...ession assays allowed to delineate
...protein* [illegible] ...ant for repression of its own promoter
... [illegible] ...ZV putative immediate-early gene
... [illegible] ...gene (ORF29) promoter. This mutational
a...lysis [illegible] ...ification of a new functional domain
...uated at [illegible] ...regions 4 and 5 which plays a crucial
... [illegible] ...nctions. This domain turned out to be
... [illegible] ...omologous alphaherpesvirus regulatory
p...teins an... [illegible] ...in bulky hydrophobic and proline
... [illegible] ...ne-rich region of the CAAT box binding
protei... [illegible] ...nofluorescence, a ***nuclear
localizat... [illegible] ...al has been mapped in region 3.

L3 ... [illegible]

AN [illegible]08 [illegible]

TI [illegible]ar l[illegible] [illegible] in the hnRNP A1 ***protein*** .

AU [illegible]; Dr [illegible]

CS [illegible] Hugh [illegible], University of Pennsylvania School [illegible]edicine [illegible]4-6148, USA..

SO [illegible] OF [illegible] May) 129 (3) 551-60.
[illegible] cod[illegible] -9525.

CY [illegible] Stat[illegible]

DT [illegible]; Ar[illegible] JOURNAL ARTICLE)

LA [illegible]

FS [illegible]

EM [illegible]

AB [illegible] (hnRNP) A1 ***protein*** is one of [illegible]jor p[illegible] mRNA [illegible] proteins in eukaryotic cells and one [illegible] most [illegible] in the nucleus. It is localized to the [illegible]plasm[illegible] also shuttles between the nucleus and the [illegible]asm. [illegible] acid sequence of A1 contains two RNP motif [illegible]nding [illegible] (RBD) the amino terminus and a glycine-rich [illegible] at t[illegible] This configuration, designated 2x [illegible], is [illegible] perhaps the largest family of hnRNP [illegible]ns. U[illegible] proteins characterized so far, A1 (and [illegible] x RBD [illegible]) does not contain a recognizable [illegible] lear* [illegible]ion*** ***signal*** (NLS). We [illegible] ound [illegible] a[illegible] 40 amino acids near the carboxyl [illegible] the [illegible] designated M9) is necessary and [illegible]ient [illegible] attachment; attaching this segment to the [illegible]ial [illegible] beta-galactosidase or to pyruvate kinase [illegible]tely [illegible] otherwise cytoplasmic proteins to the [illegible]s. Th[illegible] RNA binding motif found in the [illegible]e-ric[illegible] the R[illegible] box are not required for A1 nuclear [illegible]zatio[illegible] novel type of nuclear localization domain as [illegible]s not [illegible] similar to classical basic-type NLS. [illegible]sting [illegible] to M9 are found in other nuclear [illegible]nding [illegible] hnRNP A2.

=> d his

[illegible] 'HOME [illegible] ON 24 APR 96)

[illegible] MEDLI[illegible] AT [illegible]:54:51 ON 24 APR 96
L[illegible] 198 [illegible] R LOCAL[illegible] AND TRANSFECTION
L[illegible] 72 [illegible] LOCAL[illegible] SIGNAL AND TRANSFECTION
L[illegible] 64 [illegible] AND NUCLEAR LOCALIZATION SIGNAL AN

=> s [illegible] de or [illegible]) [illegible] localization signal and transfection

L2 363 P[illegible]
S 672 P[illegible] E
L 587 N[illegible]
L 403 L[illegible]
L 197 S[illegible]
338 N[illegible] K C[illegible] SIGNAL
[illegible] R [illegible] ON(W) SIGNAL)
48 ([illegible] C [illegible] (LA) NUCLEAR LOCALIZATION SIGNAL
L 982 T[illegible]
L4 3 ([illegible] (2A) NUCLEAR LOCALIZATION SIGNAL AND
TP[illegible]

=> d l-3 b b ab

L4 ANSWER 1 OF [illegible]
AN 95065 62 [illegible]
TI Mutational analysis of murine Mx1 protein: GTP binding core domain is essential for antiviral GTPase activity.
AU Melen K; Julkunen I
CS Molecular Biology Program, National Public Health Institute, Helsinki, Finland
SO VIROLOGY, (1995 Jan 20) 206 (1) 269-79.
Journal code: [illegible] ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9502
AB Interferon-induced resistance to influenza virus in murine cells is mediated by Mx1 protein which inhibits viral mRNA synthesis in the nucleus. The protein is a GTPase specifically targeted into the cell nucleus and has a C-terminal leucine zipper domain that mediates [illegible] oligomerization. In order to determine structural elements of the protein we created several substitution, linker insertion, and deletion mutants of murine Mx1 protein. The antiviral activity of mutant Mx1 proteins was analyzed by a transient transfection ** /influenza A infection assay in COS cells by immunofluorescence. Mx1 proteins carrying mutations in the conserved motifs within the consensus GTP binding elements exhibited significantly reduced, but not completely lost, antiviral activity against thogotovirus produced, GTP binding element substitution mutant proteins showed very low (< 10%) GTPase activity as compared to wild-type Mx1 protein. Mutations in other portions of the molecule had less effect on antiviral activity, except one mutation where we eliminated six amino acids from the C-terminal [illegible] structure evidently interrupted the

ear* al ic 1*** ***signal*** rendering
the ***prot and clearly reduced the
anti influenza of various sizes and locations
further sug terminal half of the molecule is more
important i activity than other regions of the
molecule. T indicate that the GTP binding domain of Mx1
protein is anti-influenza activity, correlating to
the GTP GTP binding element substitution
mutants, bu the molecule such as the leucine
zipper and on signal are of importance, too.

L SWE 2 OF
AN 2050 03 DLINE
TI for the activity of p53 protein.
AU Shaul ty G; Go S; Levine A J; Rotter V
CS Department Institute of Science, Rehovot,
Israel.
SO COGENE, No ( ) 55-65.
Journal code
CY ENGLAND: Un
DT Journal; Art
LA English
FS Priority Journals;
EM 01
AB p53 appears regulator, the perturbation of which
induces changes in proliferation. Wild-type p53 protein
is thought arrest gene, whereas mutant p53,
accumulates cells, has been shown to enhance
ant wild-type and mutant p53 migrate into
the cell nu identical nuclear localization signals
(NLS) inher sequences. Results presented here
show that activity of wild-type p53 measured as the
reduction primary rat fibroblasts induced by
***tra and either E1A or mutant p53, as
the ment of mutant p53 estimated by
cooperation ras formation of primary rat fibroblasts,
dependen nuclear localization signals in p53 protein. While
***transfec modified wild-type p53 significantly
reduces the fibroblast-transformed foci
induced by and ras, the wild-type p53
with lost this suppressive activity.
Pa ly p53, with a reduced nuclear
accumulation ability exhibits some suppressive activity. In
addition, coding for intact mutant p53
protein efficiently with the ras oncogene, whereas the
corresponding are totally inert. On this basis
conclude of both wild-type and mutant

p53 is a fu[illegible]mental [illegible]a[illegible] for manifesting the activities of these proteins. B[illegible] the [illegible] activity mediated by the wild-type p53 and enhanc[illegible] of [illegible] m[illegible] mediated by the mutant p53 require nuclear lo[illegible] [illegible] to function.

L[illegible] ANSWER 3 OF [illegible] MEDLINE
AN 91216452 MEDLINE
TI [illegible]rgeting the Escherichia coli lac repressor to the mammalian cell nucleus.
AU [illegible]; Dav[illegible]
CS Division of Biology, California Institute of Technology, Pasadena [illegible].
SO GENE. (1991 Mar 15) [illegible] (2) 141-5).
Journal code: [illegible]OP. [illegible] 8 2119.
CY Netherlands
DT Journal; Article; [illegible]JRNL ARTICLE)
LA English
FS Priority Journals
EM 9103
AB We have previously shown that about 90% of total Escherichia coli lac repressor synthesized [illegible] mammalian cells is located in the cytoplasm [illegible] Gene 68 (1987) 555-566]. To target a functional [illegible] to the nucleus, we mutated 10 nucleotides at the 3' end of the coding sequence, thus adding the nuclear localization signal of the simian virus 40 large-T antigen to the C terminus of the repressor. The mutant lacI gene and the wild-type (wt) gene, [illegible] in mammalian cell expression vectors, driven by the promoter of the Rous sarcoma virus long terminal repeat, were [illegible] in rodent cell lines. In confirmation of our previous [illegible] only about 10% of the wt repressor, but all of the mutant repressor [illegible] in the nucleus. DNase I footprint analyses showed that the mutant repressor retained the same operator DNA-binding specificity as the wt repressor. Furthermore, both repressor-operator complexes could be dissociated by addition of isopropyl-beta-D-thiogalactoside in vitro. However, the ratio of the number of repressor molecules per nucleus that, by in vitro activity could bind to the operator sequence to the number of monomer repressor polypeptides per nucleus, as determined by Western blotting, [illegible] about 1:3 for the wt repressor and about 1:30 for the mutant repressor. This [illegible] that: (a) the mutant repressor assembles [illegible] inefficiently; and/or (b) it has reduced binding affinity to the operator sequence; and/or (c) it has higher binding affinity to nonspecific DNA.

[illegible] nuclear lo[illegible] [illegible] and liposome

[illegible] 587 [illegible]R

6 403 ...
79197 ... AL
338 ... AR ... SIGNAL
... N(W) SIGNAL)
76 ... ME
... AND LIPOSOME

d ... an

AN ...
... 33
TI ... mediated gene transfer into ... cular tiss... by ... deficient adenovirus.
AU ... a-Walia ... Webb ... Chapman G D; Naftilan A J
CS ... Department ... University, Nashville, Tennessee, ... USA
SO GENE THERAPY ... 7128.
CY ENGLAND: Un...
LA Engli...
AB ... both in ... experiments, especially in vascular ... ls ... an e... method of gene transfer into this tissue would ... Previous methods have either yi... low ... or require complicated manipulation ... study was to develop an easy, ... plasmid DNA into vascular tissue. In ... it is demonstrated that complexing unmodified plasmid DNA ... deficient adenovirus (Ad5 dl312) via ... transfer up to 1000-fold in cultured ... (...AECs). Further, utilizing a ... model, intense nuclear ... smooth muscle cell layer and the ... transfection with a plasmid containing ... lacZ ... SV4... ***nuclear*** ***localization*** ... signal** ... demonstrated no detectable ... complexing plasmid DNA with adenoviru... enhances gene transfer both in vivo and in vi... a wide range of applications ... experim... vascular ...

s ... onic lipid?

SIGNAL)

AND CATIONIC LIPID?

transfer into vascular tissue

Chapman G D; Naftilan A J

University, Nashville, Tennessee,

ments, especially in vascular

d of gene transfer into this

Previous methods have either

require complicated manipulation

study was to develop an easy,

ified plasmid DNA into vascular

strated that complexing unmodified

ent adenovirus (Ad5 dl312) via

enhances gene transfer up to

endothelial cells (BAECs).

rabbit femoral artery model,

neointimal smooth muscle cell

ollowing transfection with a

and the SV40 ***nuclear***

. Control arteries demonstrated

suggest that complexing plasmid

l A i th ... ly enhances gene transfer both ... h ... could have a wide range of ... c t ... for expe ... ascular tissue.

= s ... y lys ... an ... a fe ... on

5 3 ... INE
1 ... L LYSINE
(... (... ,LYSINE
2 98 ... IS ... D
L ... LYS ... ID ... TRANSFECTION

= d ... b ...

I ... 3WE 1 ... 3 ...
A ... 146 39 ... MEDLINE
T ... mediated gene delivery by ...
A ... ; Huang ...
C ... Department of Pharmacology, University of Pittsburgh School of Medicine ... USA.
N ... 50 56 ... 31
S ... 1027-36.
J ...
C ... United States
D ... Journal; Article; JOURNAL ARTICLE
L ... English
F ...
E ...
A ... that several ... molecular weight cationic polymers, such as ***poly*** (... ***lysine***) and protamine, ... efficiency of several types ... -2 ... in a number of cell lines in ... spermine and a cationic decapeptide ... ly moderately active. The ... ***poly*** (... - ***lysine***) and protamine ... particle size of the complex ... DNA ... liposomes and rendered DNA resistant ... The complexes composed of DNA, ... ***poly*** ... ***lysine***), and cationic lipids were ... liposomes with sucrose gradient ... complex formed at low cationic

[illegible] pos [illegible] lipid content and only had weak
***transf[illegible] [illegible]ion of free liposome to the
[illegible] enhanced the ***transfection***
a[illegible] complexes formed at a higher initial ratio of
l[illegible] had [illegible] lipid content and were highly active in
***tr[illegible] was about 3-9-fold more active
than [illegible] before purification. Negative stain
[illegible] active complexes prepared from 40
[illegible] 0 5 [illegible] ***poly*** (***L*** -
[illegible], and [illegible] DNA and purified by gradient
u[illegible] were [illegible] electron dense, small (< 100 nm
[illegible] particles, [illegible] of them were associated with lipid
membr[illegible] stable, small-sized lipid/
po[illegible][illegible]***ly[illegible]***)/DNA complexes represent a
[illegible] delivery vehicles that might be useful in
[illegible]py

L[illegible] ANSWER 2 O[illegible] MEDLINE
AN 9[illegible]35[illegible]
TI [illegible] molecular weight glycopeptide
[illegible]
AU [illegible]; Rice K G
CS College of Pharmacy, Ohio State University, Columbus 43210, USA..
NC D[illegible]
G[illegible]
SO [illegible] 9[illegible] 6 (3) 283-91.
[illegible]2
CY [illegible]
DT [illegible]
LA E[illegible]s
FS P[illegible]
EM [illegible]
AB A [illegible] carrier was prepared by coupling
[illegible]saccharide to dp19 ***poly*** -
[illegible]g in a 1:1 conjugate. The
g[illegible] carrier [illegible] plasmid DNA as evidenced by
d[illegible]ight scattering by condensed DNA,
[illegible] on agarose gel electrophoresis.
[illegible] into HepG2 cells via the
[illegible] recognition of terminal galactose
[illegible] The resulting luciferase reporter
g[illegible] influenced by the solubility of
c[illegible] extent of [illegible]ation, and the presence of the
[illegible] The results suggest that low
[illegible] be suitable for further
[illegible] carriers for receptor-mediated gene
[illegible]

L7 [illegible]

AN [illegible]

TI [illegible] transfer to cells using a highly [illegible] biotinylated transferrin and [illegible] to streptavidin.

AU [illegible] Dawtrey A O

CS [illegible] of Medicine, University of [illegible].

SO [illegible] (6) 509-16.

[illegible]

CY [illegible]

DT [illegible]

LA [illegible]

FS [illegible]

EM [illegible]

AB [illegible] biotinylated transferrin and biotinylated [illegible]*** attached to streptavidin [illegible] transfer luciferase plasmid DNA very [illegible] presence of chloroquine. [illegible] de[illegible]on (i) use of biotinylated short [illegible] residues, (ii) biotinylated [illegible] eties, (iii) reaction of [illegible] avidin followed by isolation of [illegible] G-200 and (iv) interaction of [illegible] with biotinylated polylysine [illegible] DNA ***transfection*** . It was [illegible] steps resulting in the formation [illegible] rrin/biotinylated polylysine was [illegible] mediate conjugates by Sephadex [illegible] transfer was still very efficient. [illegible] streptavidin conjugates and [illegible] activity was almost completely [illegible], showing that gene transfer was [illegible] athway via receptor-mediated [illegible] ransferrin) bio10-pLys70 [illegible] ponents was approximately one [illegible] DNA ***transfection*** [illegible] usly described avidin-pLys460 [illegible]

L7 [illegible]

AN [illegible]

TI [illegible] warf mice with nonautologous [illegible] mate approach to somatic gene [illegible]

AU [illegible] S; Chang P L

CS [illegible] versity, Hamilton, Ontario,
[illegible]

SO [illegible], 165-75.
[illegible]03 [illegible]

CY [illegible]

DT [illegible]

LA [illegible]

FS [illegible]

EM [illegible]

AB [illegible] gene therapy protocols depend [illegible] cells. We propose an alternate [illegible] approach by implanting [illegible] lines to deliver desired gene [illegible]. The recombinant allogeneic [illegible] after implantation by enclosure [illegible] ***poly*** - ***L*** - [illegible]. The clinical efficacy of this [illegible] implanting microencapsulated [illegible] secrete mouse growth hormone into [illegible] dwarf mice. The treated mutants [illegible] body weights, peripheral organ [illegible] kness significantly greater [illegible]. Secondary response to the [illegible] also resulted in increased [illegible] month post-implantation. The [illegible] months of implantation [illegible] blasts retained a viability of [illegible] growth hormone. Thus, [illegible] binant cells corrected partially [illegible] iption factor mutation in the [illegible] cells remained functional for [illegible] of delivery recombinant gene [illegible], obviates the need for [illegible], and is amenable to [illegible] should have wide applications [illegible] ntinuous supply of recombinant [illegible]

L7 [illegible]

AN [illegible]

TI [illegible] um of animals by targeting the [illegible]

AU [illegible] el C S; Hanson R W; Davis P B

CS [illegible] es and Childrens Hospital, [illegible]

NC [illegible]

SO [illegible] 95 Feb) 95 (2) 493-502.
[illegible] 1-9

CY [illegible]

DT [illegible]

LA [illegible]

FS [illegible] rity Journals; Cancer Journals

EM [illegible]

AB [illegible] specifically to respiratory [illegible] with high efficiency by [illegible] adocytosis of the polymeric [illegible] carrier, consisting of the Fab [illegible] d against rat secretory [illegible] ***poly*** - ***L*** - [illegible] plasmids containing different [illegible] cells in vivo. We observed [illegible] me activity in protein extracts [illegible] imum values of 13,795 +/- [illegible] ed light units (ILU) per [illegible] vely. No luciferase activity [illegible] do not express the receptor. [illegible] ting of an irrelevant plasmid [illegible] carrier or the expression plasmid [illegible] on an irrelevant Fab fragment [illegible] erase activity in all tissues [illegible] ain cells bearing the [illegible] transfected, and [illegible] uted to the nonspecific uptake [illegible]. Specific mRNA from the [illegible] the lungs of transfected [illegible] he lungs are transfected by [illegible] ed containing expression [illegible] rial beta-galactosidase or the [illegible] on of these genes was [illegible] of the airways and the [illegible] ioles and alveoli. [illegible] used to introduce functional [illegible] of rats, and may be a useful [illegible] ne lung.

L7 [illegible]

AN [illegible]

TI [illegible] ransfer to human T leukaemic [illegible]

AU [illegible] er K; Rusconi S; Dinter A; [illegible]

CS [illegible] Zurich, Switzerland..
SO [illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] antigen (GalNAc alpha
[illegible] D-galactosamine) is expressed
[illegible] disorders including the Tn
[illegible] virus (HIV) coat
[illegible] normal, differentiated cells
[illegible] processing galactosyltransferase.
[illegible] express high levels of Tn
[illegible] ation, we have established the
[illegible] demonstrate the considerable
[illegible] ***poly*** (***L*** -
[illegible] monoclonal antibody 1E3 directed
[illegible] luciferase and
[illegible] Jurkat cells by
[illegible] of unconjugated 1E3 reduced
[illegible] concentration-dependent manner
[illegible] shed DNA transfer completely,
[illegible] mediated by the Tn antigen.
[illegible] Vibrio cholerae sialidase, which
[illegible] ulted in an improvement of gene
[illegible] chicken adenovirus particles
[illegible] strongly augmented transgene
[illegible] (lacZ) gene was delivered to
[illegible] is, up to 60% of the cells were
[illegible] ing 5-bromo-4-chloro-3-indolyl-
[illegible] chromogenic substrate. The
[illegible] -mediated DNA uptake into
[illegible] though these cells were shown
[illegible] nsferrin receptor. We show
[illegible] antigen mediates highly
[illegible] into Jurkat T cells. This method
[illegible] Jurkat cell ***transfection***
[illegible] transfer techniques. Specific
[illegible] exhibiting Tn epitopes may
[illegible] protocols.

L7 [illegible]
AN [illegible]
TI [illegible] *** - ***L*** -
[illegible] gene delivery.

AU [illegible] Chio A F; Armendariz-Borunda J;
[illegible]
CS [illegible] Veterans Affairs Medical Center,
[illegible]
SO [illegible] 2 [illegible]
[illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] delivery relies on the formation
[illegible] molecule that will be
[illegible] cells. In the case of
[illegible] convenient targeting molecules
[illegible] idity of the hepatocyte galactose
[illegible] - ***L*** - ***lysine***
[illegible] protein, and the resulting
[illegible] sion vector (DNA). The
[illegible] ***poly*** - ***L*** -
[illegible] ures delivery of the
[illegible] liver, where it is subjected
[illegible] , the ***poly*** - ***L***
[illegible] complexes tend to be unstable,
[illegible] bohydrate content. For these
[illegible] rnative. We exploited the
[illegible] be reductively coupled to the
[illegible] used lactose to obtain
[illegible] with "exposed" galactose.
[illegible] ide at high pH in borate
[illegible] edure. The "lactosylated"
[illegible] has proved very stable,
[illegible] asmids. In a set of experiments
[illegible] - ***L*** -
[illegible] lactosylated ***poly*** -
[illegible] exes by transfecting hepatoma
[illegible] experiments we used a
[illegible] on vector containing a mutant
[illegible] TRUNCATED AT 250 WORDS)

L7 [illegible]
AN [illegible] I
TI [illegible] fibroblasts expressing the
[illegible]
AU [illegible] C; [illegible] ang F
CS [illegible] tat Tubingen, Germany..
SO [illegible] Apr) 63 (2) 316-25.

CY GERMANY

DT

LA

FS

EM

AB

er NIH 3T3 fibroblasts leads to a
on and causes an increase in
exchange and Na+, K+, 2Cl-
systems are thought to be
of this study was to examine
ics and cytoskeletal
he oncogene. The experiments
ransfected with a
and expressing the oncogene
and 1 mumol/l dexamethasone
expressing the oncogene (-ras
dium, but without the addition
The growth characteristics
itecture was visualized by
using specific antibodies and
as oncogene was accompanied by
crease in proliferative
ating of the dishes with attachment
lysine , collagen type
ated slower on substrates
lysine than on
pe I. Expression of the ras
icant increase in cell volume
te. +ras Cells became more
protrusions and tended to
Examination of the
ras cells revealed marked
on of the stress fiber network
well as the absence of
(focal contacts), a
nd of cell surface fibronectin
, re ion of microtubules and
egion was also observed in
broblasts which were not
as cells) and which were also
ditions described above (low serum
exhibit the cytoskeletal
The results demonstrate that
ot only profound alterations
ume and cell morphology, but
keletal architecture, which may

I [illegible] of volume-regulatory ion
t [illegible]

L7 [illegible]
AN [illegible]
TI [illegible] cells through use of
a [illegible] to biotinylated transferrin
a [illegible]
AU [illegible] B; Ariatti M; Hawtrey A
CS I [illegible] of Medicine, University of
[illegible]
SO [illegible] (2) 165-74.
[illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] for studying the transfer of
I [illegible] eptor-mediated endocytosis.
[illegible] 460 was covalently
a [illegible] of avidin via periodate
c [illegible] avidin-pLys460. Following
p [illegible], the conjugate was reacted with
b [illegible] ting avidin-pLys460-[bio-
t [illegible] ated by Superose 12 gel
c [illegible] periments with DNA.
I [illegible] n avidin-pLys460-]bio-
t [illegible] t by nitro cellulose filter
b [illegible] assays. The conjugate was shown
t [illegible] complexes soluble in 0.15-0.2 M
[illegible] avidin-pLys460-[bio-transferrin]
a [illegible] accomplished with Hela cells,
a [illegible] lanoma cell line used in the
[illegible] was dependent on
[illegible] lysosomotropic agent
[illegible] ent with a receptor-mediated
e [illegible] v for Hela cells and a
c [illegible] endocytosis for the alpha T3
[illegible] r

L7 [illegible]
AN [illegible]
TI [illegible] of an adenovirus-based ligand
c plex for gene transfer.
AU [illegible] J; [illegible]
CS [illegible] gineering, University of

I [illegible], Philadelphia..
SO [illegible] Apr [illegible] (Pt 1) 49-58.
[illegible]nal code: 9YO. ISSN: 0264-602[illegible].
CY [illegible]: United Kingdom
DT Journal Article; JOURNAL ARTICLE
LA English
FS [illegible] Journal [illegible]
EM [illegible]
AB [illegible]and-mediated approaches to gene transfer offer an alternative to viral vectors for [illegible] in vivo and in vitro applications. Although a significant proportion of the plasmid-based DNA complex is lost to lysosomal degradation following receptor-mediated endocytosis, simultaneous infection with adenovirus has been shown to increase the [illegible] of [illegible] (Curiel, Agarwal, Wagner and Cotten 1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8850-8854; Wagner, Zatloukal, Cotten, Kirlappos, Mechtler, Curiel and Birnstiel (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 6099-6103]. In this study we describe an adenovirus-based ligand complex where the plasmid DNA, polycation-ligand conjugate and adenovirus are contained within a single [illegible]. As a model of the ***transfection*** procedure, a replication deficient recombinant adenovirus encoding a cDNA antigene for human placental alkaline phosphatase that was chemically modified with ***poly*** (***L*** - ***lysine***) A[illegible]s). Electron microscopy of an adenovirus-based ligand complex formed by [illegible] plasmid DNA and an [illegible] ***L*** - ***lysine***) conjugate revealed [illegible] structures that appeared as intact viral particles coated with a dense biomolecular layer. Adenovirus-based ligand complexes containing either a luciferase or beta-galactosidase reporter plasmid were shown to efficiently deliver the plasmid transgene to cells that express the hepatic asialoglycoprotein receptor. Furthermore, the ***poly*** ([illegible] modification greatly reduced the infectivity potential of the virus without causing a concomitant loss of augmented gene transfer. As an alternative to infectious virions, incomplete products of viral assembly were also considered as a source of [illegible] activity. However, these defective virions were unable to significantly enhance plasmid transgene [illegible]

L7 [illegible] OF 28 MEDLINE
AN [illegible] MEDLINE
TI [illegible] and uptake in vitro receptor-mediated ***transfection***
AU [illegible] Wagner E J
CS [illegible] University of California-Davis..
SO [illegible] JOURNAL OF THE MEDICAL SCIENCES, (1994 Feb) 307 (2) 138-43.

[illegible] ode [illegible] 002-9[illegible]
CY U[illegible] ate[illegible]
DT [illegible] Art[illegible]e [illegible]
LA E[illegible]
FS [illegible] Index Med[illegible] Journals; [illegible]y Journals
EM [illegible]
AB [illegible] efficien[illegible] specific ***transfection*** by [illegible]ned [illegible]ed [illegible] by the use of cationic lipids. A[illegible]op[illegible]e [illegible]ated to ***poly*** - ***L*** - [illegible]ine [illegible] with the plasmid pCMVL that [illegible] lucifera[illegible] gene. The asialoglycoprotein- ***po[illegible]*** - ***L[illegible]*** - ***lys[illegible]*** :pCMVL (AP-PL:pCMVL) complex [illegible] the[illegible] xed with [illegible] cationic lipid [illegible] This complex was taken up by [illegible]ocy[illegible] via the asialoglycoprotein [illegible]. Th[illegible] x[illegible] in cells transfected with the [illegible] were significantly increased compared [illegible] exes witho[illegible] The ratio of AP-PL to DOGS i[illegible] an important de[illegible]nant for [illegible] ***transfection*** e[illegible] eptor specificity. Therefore, c[illegible] lip[illegible] eased the efficiency of a[illegible]ycop[illegible]e [illegible] ***transfection*** in the h[illegible] astom[illegible] Hep[illegible]. The use of cationic lipids with [illegible] delivery s[illegible] could potentially increase ***transfection*** efficiency [illegible] maintain cell-target s[illegible].

L7 [illegible]
AN [illegible]
TI [illegible] gene [illegible] delivery and expression in primary hepatocytes [illegible]izing a [illegible]ated adenovirus-DNA complex.
AU C[illegible] Kay M [illegible] Brinkley B R; Woo S L
CS [illegible] Hughes Medical Institute, Baylor C[illegible] 77030.
NC [illegible] (NI[illegible])
[illegible] NH[illegible]
[illegible] (NC[illegible])
SO [illegible] SCIENCES OF THE UNITED STATES O[illegible] 1548-52.
[illegible] code [illegible]
CY [illegible] State[illegible]
DT [illegible] JR[illegible]
LA E[illegible]
FS [illegible] Jou[illegible]
EM [illegible]
AB [illegible] effective method for gene

[illegible] cells. We [illegible] previously shown that DNA
[illegible] protein can be efficiently
[illegible] the internalized DNA can be
[illegible] a replication-defective
[illegible] enter target cells
[illegible] requires high concentrations of
[illegible] adenoviral particles were
[illegible] to ***poly*** (***L*** - ***lysine***
) [illegible] Quantitative delivery to
[illegible] significantly reduced viral
[illegible] ***poly*** (***L*** -
[illegible] in the complex. The
[illegible] er a DNA vector containing
[illegible] use hepatocytes, resulting in the expression
[illegible] factor IX in the culture
[illegible] receptor-mediated endocytosis
[illegible] lysis vector should permit the
[illegible] gene delivery into the liver
[illegible] deficiencies.

L7 [illegible]
AN [illegible]
TI [illegible] specific for G-protein coupled [illegible] tope tagged BLR1-transfected [illegible] ELISA
AU [illegible] Voss C, [illegible]
CS [illegible] der [illegible], Martinsried, FRG..
SO [illegible] COMMUNICATIONS, (1993 Nov 15) [illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] protein coupled receptors are [illegible] ELISA-based screening system [illegible] the G-protein coupled [illegible] BLR [illegible] Immunol. [illegible] :2795) using human embryonic [illegible] modified human BLR1 cDNA [illegible] tagged BLR1 protein. Lou/C [illegible] ed, tagged 293 cells and [illegible] 8.653 myeloma cells [illegible] specific antibodies by comparing [illegible] s and to untransfected [illegible] ***L*** - [illegible]. Cells were fixed with 2%

p[illegible] digitonin in order to allow
b[illegible] intracellular epitopes. This mild
f[illegible] morphology of 293 cells and allowed
r[illegible] sorting of approximately 2500
s[illegible] binding to BLR1 transfected
2[illegible]. One of these mAb
s[illegible] coupled receptor BLR1.

L7 A[illegible] OF[illegible]
AN 9[illegible]
TI P[illegible] using stable
m[illegible] *** - ***L*** - ***lysine***
c[illegible] Anal Biochem 1993 Nov
1[illegible]
AU [illegible]
CS D[illegible]ogy, Pennsylvania State
U[illegible]
NC F[illegible]
F[illegible]
SO A[illegible]13 (1) 97-103.
C[illegible]
CY U[illegible]
DT J[illegible]
LA E[illegible]
FS E[illegible]
EM 9
AB C[illegible] provide favorable routes for
D[illegible] target cell specificity, transfer
e[illegible] transferrin receptor system
a[illegible]fection*** is developed here
v[illegible] of DNA to stable
m[illegible] *** - ***L*** - ***lysine***
c[illegible] importance of precise
s[illegible] ***poly*** - ***L*** -
[illegible] chemistry for their
c[illegible] quantitatively are effects
c[illegible]on the efficiency of
t[illegible] : lysine polymer length,
c[illegible]ratio, and treatment of
t[illegible]ferrioxamine. Compared directly
t[illegible], modified DEAE-dextran,
l[illegible]ols), ***transfection***
v[illegible] system was > or = 10-fold
m[illegible]ic to erythroleukemic F-MEL
a[illegible]on*** these cells retained
t[illegible]induced differentiation in
[illegible]cells), or to erythropoietin

...lator of erythropoiesis. Thus,
...eceptor-mediated
... should be widely applicable
... systems both in vitro and in
...).

L7 A... OF ...
AN ...
TI M... ce...: a new delivery system for
...
AU T... D... Su...
CS D... ...y ...v... ...f Toronto, Ontario, Canada..
SO F... ...RN... ...).
...
CY U... ...
DT ...; ...RNA... ...B)
LA E...
FS P... To... ...
EM ...
AB T... ...ene therapy has been
ex... ...s, yet its slow progression
i... ...uted to problems associated
v... ...t gene transfers, to obtain
s... ...he transfected gene, and the
r... ...er transplantation. Here we
i... ...e therapy that overcomes all
th... ...; ge...tically modified cells in a
b... ...ducing a system that can
p... ...e desired gene product. As a model,
m... ...human growth hormone gene
(... ...inate- ***poly*** - ***L***
-... ...ong-term in vitro studies
s... ...ells was physiologically
co... ...the cells. Furthermore, there
w... ...the human protein by the
e... ...of steady increase in the
s... ...by each cell, followed by a
p... ...lence of the feasibility of
t... ...vo study: Balb-c mice
tr... ...-G cells had detectable serum
l... ...or the duration of the study
(... ...ls recovered from a recipient
1... ...nued to secrete high levels of
h...

L7 A... ...
AN ... ...

TI [illegible] cement of receptor-mediated [illegible] y hepatocytes.

AU [illegible]

CS [illegible] lege of Medicine, Houston, TX [illegible]

NC [illegible]

SO [illegible] SCIENCES OF THE UNITED STATES [illegible]

CY [illegible]

DT [illegible]

LA [illegible]

FS [illegible]

EM [illegible]

AB [illegible] NA delivery system with the [illegible] and shown that DNA can be [illegible] sulting in a high level of [illegible] d conjugated with ***poly*** [illegible] d to deliver the Escherichia [illegible] ary hepatocytes through [illegible] otein receptor, only a low [illegible] able, with less than 0.1% of [illegible] s level of activity can be [illegible] ion of the DNA.protein complex [illegible] s, resulting in 100% of the [illegible] -4-chloro-3-indolyl [illegible] sis of beta-galactosidase [illegible] ancement of activity. To test [illegible] system for the correction of [illegible] that causes severe mental [illegible] ered the human phenylalanine [illegible] derived from a PAH-deficient [illegible] reconstitution of enzymatic [illegible] ise for efficient gene [illegible] f hepatic disorders.

L7 [illegible]

AN [illegible]

TI [illegible] tosylated ***poly*** - [illegible] ma cells.

AU [illegible] J; Mayer R; Monsigny M; Roche [illegible]

CS [illegible] ugues et Lectines Endog`enes, [illegible]

SO [illegible] 21 (4) 871-8. [illegible]

CY E

DT J

LA E

FS P s

EM 9

AB P mplexes were used to transfer
i to human hepatoma cells by a
r HepG2 cells which express a
g e efficiently and selectively
t polylysine complexes in a
s which do not express membrane
l poorly transfected with
p xes, ii) HeLa cells which do
n or galactose were not
t polylysine complexes. The
* G2 cells with
p xes was greatly enhanced
e or in the presence of a
f le derived from the influenza
v ypeptide that mimics the
f elected. In the presence of
t activity in HepG2 cells was 10
f cted with pSV2Luc/lactosylated
p of chloroquine.

L7 A

AN 9

TI T pression of hepatitis B viral
D a

AU L a K; Zhang Y; Wands J R; Wu C
H

CS C s, Massachusetts General
F

NC D
C
C
+

SO J 93 Mar) 91 (3) 1241-6.
J

CY U

DT J

LA E

FS A ity Journals; Cancer Journals

EM 9

AB A g of an asialoglycoprotein
c ***L*** - ***lysine*** was
us B virus (HBV) DNA constructs

t … e human hepatoma cells. 4 d
a … ace or core gene expression
c … a were measured to be 16
r … pectively. Antigen production
w … on of an excess of
a … asialoglycoprotein
r … SK-Hep1, did not produce any
v … ons after incubation with HBV
D … of asialoorosomucoid and
* … . Using a complete HBV
g … s reached 16 ng/ml and 16
U … thern blots revealed
c … luding 3.5-, 2.4-, and 2.1-kb
f … ular HBV DNA sequences
i … single stranded forms were
c … n. Finally, 42-nm Dane
p … res medium were visualized by
e … rates that a targetable DNA
c … n vitro resulting in the
p …

L7 A …
AN …
TI C … ivery and expression of
e … odified ***poly*** (
* … onjugate in mouse lung
e …
AU T … ; Huang L
CS D … of Tennessee, Knoxville..
NC A …
SO … .C(HY … A … ul 15) 1131 (3) 311-3.
… -3 …
CY N …
DT C … f …
LA E …
FS …
EM …
AB A … gene delivery and expression
i … fficiency of N-terminal
m … **lysine***) (NPLL)
c … ibody 34A can be improved by
a … ment, cationic liposomes.
D … posomes form a ternary
e … omple … the ability to bind
s … e same time the addition of
l … nsfection*** efficiency of
a … by 10 to 20-fold in mouse

L7 A
AN 9
TI T and sperm cells.
AU I ; Spadafora C
CS D iversity La Sapienza, Rome,
I
SO , (1992 Mar) 31 (3) 161-9.
CY
DT
LA
FS
EM 9
AB plasmid DNA, showed a
the exogenous nucleic acid. We
of such interaction. Exogenous
15- to 20-min period and is
area of the sperm head. DNA
nce it can be competed out by
her polyanions as heparin and
L -
the uptake. DNA molecules of
aken up as compared to
ins were also taken up and
r level. These data strongly
occur between foreign
e sperm head. On the basis of
otein(s) of 30-35 KD is
exogenous DNA binding.
asma contains factor(s) which
powerful inhibitor effect on
binding protein for the DNA
ion support the existence of
lc factors, the sperm-DNA

L7 A
AN 9
TI ar transport of a
t
AU ; Serebryakova N V; Murav'ev
V
CS ity of Health, Moscow..
SO 99 (2) 323-9.

CY U
DT C
LA E
FS F
EM S
AB A ...smid carrying the gene of the
S ... ***poly*** - ***L*** -
* ... lectively transfect PLC/PRF/5
h ... lin receptors.
* ... y competed by excess free
i ... port of the construct, it was
f ... ion on and in cells
v ... and quantitative confocal
l ... 37 degrees C, the labeled
c ... ntracellular acidic
c ... on of fluorescence localized
b ... ing, endocytosis, and nuclear
l ... ould all be competed by
e ... at entry of the conjugate
i ... the insulin receptor.

L7 A 2
AN S
TI D ... functional gene-delivery
a ... ne or ethidium homodimer to
t
AU W ... pos H; Birnstiel M L
CS F ... ogy, Vienna, Austria..
SO E ... 2 (4) 226-31.
C
CY U
DT C
LA E
FS F
EM S
AB W ... ansferrin-polycation
c ... es for the introduction of
g ... here a more specific method
f ... -binding compounds involving
a ... y ate moiety. We used the
p ... ***lysine***) or the DNA
i ... DNA-binding domains. Successful
t ... nd expression of the
I ... cells has been shown with
t ... ectivity of the
t ... conjugates is low relative to
t ... ably because of incomplete

c [illegible] delivery with TfEtD is
c [illegible] xes of the DNA with TfEtD
a [illegible] e are prepared. The gene
c [illegible] transferrin-polylysine
c [illegible] scribed conjugates containing
c [illegible] thod facilitates the synthesis
c [illegible] of conjugates.

L7 [illegible]
AN [illegible]
TI I [illegible] poly [illegible] DNA ***transfection***
[illegible]
AU Z [illegible]
CS D [illegible] of Tennessee, Knoxville
[illegible]
SO [illegible] ay 31) 1065 (1) 8-14.
[illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] 3000) ***poly*** (
[illegible] ugated to N-
[illegible] effective carrier to promote
[illegible] ultured mammalian cells. The
[illegible] ontained an average of two
[illegible] L. Similar conjugates of the
[illegible] a similar
[illegible] ing that the degradation of
[illegible] tivity. Unconjugated
[illegible] ***transfection*** activity
[illegible] ed with respect to the DNA
[illegible] bation time and the presence
[illegible] binding of lipopolylysine
[illegible] retardation of DNA in agarose
[illegible] at the optimal
[illegible] sfection*** , all DNA were
[illegible] nter the gel. The
[illegible] ipopolylysine, under optimal
[illegible] igher than that of lipofectin,
[illegible] over, lipopolylysine mediated
[illegible] ne of 10% calf serum; whereas
[illegible] tivity under the same
[illegible] ***transfection***
[illegible] d on scraping the treated
[illegible] transfected attached

L7 [illegible]
AN [illegible]
TI [illegible] expression of a foreign ge[illegible] ements in vivo.
AU [illegible] M [illegible]
CS [illegible] nnecticut School of [illegible]
NC [illegible]
SO [illegible] Oct 15) 264 (29) 16985-7.
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM 9[illegible]
AB [illegible] e driven by natural mammalian [illegible] hepatocytes and the resultant [illegible] was accomplished using a [illegible] of two covalently linked [illegible] *** - ***L*** - [illegible] strong but non-damaging [illegible] n, [illegible] ein which can be targeted [illegible] ly [illegible] rface asialoglycoprotein [illegible] asmid, palb-CAT, containing [illegible] nsferase (CAT) driven by mouse [illegible] exed to the carrier system. [illegible] in the form of a complex [illegible] activity in liver homogenates [illegible] e expression, however, was [illegible] g/g liver at 24 h but was [illegible] hepatectomy 30 min after [illegible] levels of hepatic CAT activity [illegible] injection. Southern analysis of [illegible] 1 we [illegible] ony demonstrated that some of [illegible] ed [illegible] nto the host genome. We [illegible] ha [illegible] atural mammalian regulatory [illegible] ar [illegible] by intravenous injection in [illegible] foreign gene expression [illegible] persist by stimulation of [illegible]

L7 [illegible] OF [illegible]
AN [illegible]
TI [illegible] ession in vivo.

AU W[illegible] C H

CS D[illegible] of M[illegible] Connecticut, School of M[illegible] Fa[illegible]

NC C[illegible]
C[illegible]

SO C[illegible] [illegible] G[illegible] Oct 15) 263 (29) 14621-4.
C[illegible] de: H[illegible]

CY U[illegible] te.

DT C[illegible] rt[illegible] C[illegible]

LA E

FS E[illegible]

EM 8

AB A[illegible] DNA c[illegible] to target a foreign gene s[illegible] ly to l[illegible] loglycoprotein receptors. The D[illegible] a galactose-terminal [illegible] ly [illegible] ii (AsOR), covalently linked t[illegible] ly[illegible] ive*** . The conjugate was c[illegible] on AsOR content of the c[illegible] to t[illegible] ontaining the gene for the b[illegible] enzym[illegible] ltransferase (CAT). I[illegible] DNA complexed to the carrier c[illegible] with 85% of the injected c[illegible] compared to only 17% of the c[illegible] alone was injected under i[illegible] DNA was detected at a level c[illegible] a [32P]pSV2 CAT cDNA probe to 1[illegible] ravenous injection of AsOR- [illegible] -DNA complex containing 1.0 m[illegible] h after injection of the c[illegible] gene was functional as 1[illegible] ty (approximately 4 m[illegible] ntrol animals that received [illegible] produced no CAT activity. [illegible] to compete with the AsOR- [illegible] -DNA complex for uptake by t[illegible] Assays for CAT activity in c[illegible] filed to demonstrate any a[illegible] ble DNA carrier system can [illegible] nes specifically to liver with 1[illegible] vo.

L7 [illegible]

AN 8[illegible]

TI [illegible] Hep G2 hepatoma cells in [illegible]

AU [illegible]

CS [illegible] Connecticut School of

SO [illegible] 88 2.

CY [illegible] tes

DT [illegible] rti

LA [illegible]

FS [illegible]

EM [illegible]

AB [illegible] ng foreign DNA to hepatocytes [illegible] nat takes advantage of [illegible] edi [illegible] ve internalization. The idea [illegible] possess a unique receptor that [illegible] al (asialo)glycoproteins. To [illegible] could bind DNA in a [illegible] *** (***L*** - [illegible] but noncovalent interaction. [illegible] yc [illegible] d (AsOR), was chemically [illegible] ***lysine***) to form an [illegible] *** - ***lysine***) [illegible] gate to DNA were tested to [illegible] content in a soluble complex [illegible] s. To test the targetable [illegible] (***L*** - ***lysine***) [illegible] e was [illegible] d pSV2 CAT containing the [illegible] ase (CAT) driven by an SV-40 [illegible] a model system consisting of [illegible] glycoprotein receptor (+)], [illegible] and uterine smooth muscle [illegible] s incubated with 0.2 micron [illegible] SOR [illegible] - ***lysine***)-DNA [illegible] lus AsOR, DNA plus [illegible]), or DNA alone. Cells were [illegible] y as a measure of gene [illegible] mooth muscle [receptor (-)] [illegible] chloramphenicol derivatives [illegible] TRUNCATED AT 250 WORDS)

L7 [illegible]

AN [illegible]

TI [illegible] side chain lengths and of [illegible] cs of basic [illegible] mate cells to [illegible]

AU [illegible]

SO [illegible] 4.

CY [illegible]

DT
LA
FS
EM
AB cell sheets to
was determined for 5 basic
trobromide at 100 microng/ml,
for poly-L-ornithine of
nan of AMW 105000, and much
lysine of AMW 1700
ses were observed with the 2
much faster and sensitivity
polyornithines than for the
0 sensitized cells slightly
- ***lysine*** of AMW
ivity maximum. Analysis of the
*** ***L*** - ***lysine***
step 1 (attachment) and a

L7
AN
TI ured mammalian cells. Binding
AU
SO .e 13) 454 (3) 397-409.
CY
DT
LA
FS
EM
AB were incubated for a short
ial DNA and
n), ***poly*** - ***L***
e, agents previously
nce ***transfection*** .
y 10(-6)-1.5 by 10(6) cells
r DNA representing 10-30% of
In contrast, when the cells
solution approx. 0.5-5% of the
ore than 95% of the
be bound to the surface of
lso shown that in the absence
ll-associated donor DNA was

=> d h

APR 96)

24 APR 96
L1 TRANSFECTION
L2 AL AND TRANSFECTION
L3 NUCLEAR LOCALIZATION SIGNAL AN
L4 NUCLEAR LOCALIZATION SIGNAL A
L5 AL AND LIPOSOME
L6 AL AND CATIONIC LIPID?
L7 CTION

=> s p

Y
3
2

L8

=> d 1 bi

L8
AN
TI *** -mediated gene delivery
AU
CS of Pittsburgh School of
USA
NC
SO 7-3
CY
DT AP ICI
LA
FS
EM
AB lar weight cationic polymers,
** ysine***) and protamine,
on ffi / of several types of cationic

[illegible] cell lines in vitro. Small
[illegible] ionic decapeptide derived from
[illegible] tive. The addition of
[illegible] ** lys [illegible] and protamine
[illegible] of the complex formed between
[illegible] ed DNA resistant to the
[illegible] sed of DNA, ***poly*** (
[illegible] nic lipids were purified from
[illegible] gradient
[illegible] complex formed at low cationic
[illegible] lipid content and only had weak
[illegible] ***liposome*** to the
[illegible] d the transfection activity.
[illegible] her initial ratio of
[illegible] lipid content and were highly
[illegible] tiv [illegible] as about 3-9-fold more active
[illegible] purification. Negative stain
[illegible] ve complexes prepared from 40
[illegible] poly*** (***L*** -
[illegible] and purified by gradient
[illegible] ectron dense, small (< 100 nm
[illegible] were associated with lipid
[illegible] small-sized lipid/
[illegible])/DNA complexes represent a
[illegible] hicles that might be useful in
[illegible]

L8 [illegible]
AN [illegible]
TI [illegible] bilization by polycationic
[illegible]
AU [illegible]
CS [illegible] niversity Health Sciences
[illegible]
SO [illegible] 15 [illegible] 51 50.
[illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] akage and fusion of liposomes
[illegible] estigated the nature and
[illegible] ical properties caused by
[illegible] in the observed membrane
[illegible] range of pH 5 to pH 7 both
[illegible] ***L*** - ***lysine***

...er to hexagonal phase
...osphatidylethanolamine, either
...yl-2-
...ted the gel to liquid
... of 1:1 mixtures of
...anolamine, both in dimyristoyl
...oyl forms, as a function of pH
...ycationic amino acids. We
...gely miscible at all pH
... of the polypeptides. However,
...phase separation at higher pH
... neither changes in curvature
...duced by the polycationic
...ked ability to induce leakage
...abeled with pyrene on one of
... emission from both monomer
...on intensity from these two
...eparation and the degree of
...imolar mixtures of the
...idylserine and
... crystalline phase at 30
... the ratio of excimer to
...***poly*** - ***L*** -
...ction of excimer emission from
...om 5 to 7. Poly-L-histidine
...ratio at pH 5 but not at pH
...expert for lateral phase
..., the consequence of the
... diffusion of the lipids.
...rved over a range of
... both gel and liquid
...e from the behaviour of the
...ordigitation.(ABSTRACT

L8 ...

AN ...

TI ...ose oxidase electrodes for the
...

AU ...rson, S P

CS ...ty of Manchester, UK..

SO ... (3-4) 251-60.

CY ...

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LA ...

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EM

AB ... the measurement of glucose ... Glucose oxidase has been used ... sensors but the performance ... duration kinetics, which ... relevant glucose ... limiting membranes have been ... of the enzyme to lower ... sent in the bulk test ... electrode was reported whereby ... liposomes so that the lipid ... brane. It was shown that the ... lipid constituents of the ... sponse to glucose could be ... bes research undertaken to ... liposomal enzyme electrode. ... demonstrated with the use of ... solution. The variation in ... amount of glucose oxidase ... The new method allows a ... from a single batch of ... ling effects of the lipid ... he response of the electrode ...

L8 ...

AN ...

TI ... by ***poly*** (***L*** ... ***liposome*** encapsulation.

AU ... Chaud P; Lebleu B

CS ... Universite Montpellier II ... Langue ... rance..

SO ... '1 ... 1; 81-94. Ref: 89

CY ...

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FS ...

EM ...

=> S ...

L9 [illegible] IPTD?

=> d [illegible]

L9 [illegible]
AN [illegible]
TI [illegible] gene delivery by [illegible]
AU [illegible]
CS [illegible] of Pittsburgh School of [illegible]
NC [illegible]
SO [illegible] 27-36.
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
EM [illegible]
AB [illegible] ular weight cationic polymers, [illegible] ***lysine***) and protamine, [illegible] several types of cationic [illegible] cell lines in vitro. Small [illegible] ionic decapeptide derived from [illegible] moderately active. The addition of [illegible] **) and protamine [illegible] of the complex formed between [illegible] ed DNA resistant to the [illegible] composed of DNA, ***poly*** ([illegible] cationic*** ***lipids*** [illegible] liposomes with sucrose gradient [illegible] formed at low cationic [illegible] ent and only had weak [illegible] ee liposome to the purified [illegible] nsfection activity. In [illegible] ed at high initial ratio of liposome to [illegible] highly active in [illegible] 3-fold more active than the

Negative stain EM studies
epared from 40 nmol of
L - ***lysine***
gradient
electron dense, small (< 100 nm
were associated with lipid
ble, small-sized lipid/
/DNA complexes represent a
es that might be useful in

L9
AN
TI ween ***cationic***
and DNA
AU J; Graham R W; Bally M B
CS Columbia Cancer Agency,

SO

CY
DT
LA
FS
EM
AB d-based carrier system for
the characterization of
ns and carrier formation. We
mplex can be formed following
lipids*** to DNA in a Bligh
roform/methanol/water
the monophase into a two-phase
to the organic phase. When
lipids , such as
ioleyldimethylammonium
aminopropane chloride,
can be recovered in the organic
ntrations sufficient to
the polyvalent
dioleyloxy-N-
thy - 1- propanaminium
spermidine are used,
the organic phase is also
lipid and DNA is
hydrophobic DNA complex can
lipids . In the
of excess Ca2+,

...***lysine***), 100% of
... The monovalent
...xes can also be prepared
... concentrations of NaCl (<
... Importantly, these
...***cationic*** ***lipid***
... The methods described,
... characterized in the
... ACT LIMITED AT 250 WORDS)

L9 ...

AN ...

TI ... c ... in vitro

AU ... s

CS ... California-Davis..

SO ..., (1994 Feb) 307 (2) 138-43.

CY ... s

DT ...

LA ...

FS ... Journals

EM ...

AB ...ection by receptor-mediated
...***cationic*** ***lipids***
... ***poly*** - ***L***
... the plasmid pCMVL that
... ialoglycoprotein-
... :pCMVL (AP-PL:pCMVL)
...***cationic*** ***lipid***
... complex was taken up by
... the asialoglycoprotein
... ells transfected with the
...ficantly increased compared
... The ratio of AP-PL to DOGS
...sfection efficiency and for
..., ***cationic***
... efficiency of
...sfection in the
... of ***cationic***
...e delivery systems could
...ency yet maintain

=> s ...

...A

L10 [illegible] FECTION

=> s [illegible] in and transfection

[illegible] DNA BINDING DOMAIN

L11 [illegible] BINDING DOMAIN AND TRANSFECT

=> d [illegible]

L11 [illegible]
AN [illegible]
TI [illegible] transcriptional repressor [illegible] rigenesis and is regulated [illegible] togenic stimulation.
AU [illegible] Jr; Fisher R J; Blair D G;
CS [illegible] onal Cancer Institute,
SO [illegible]
CY [illegible]
DT [illegible]
LA [illegible]
FS [illegible]
OS [illegible]
EM [illegible]
AB [illegible] member of the ets family of [illegible] its interaction with the ets [illegible] romoter. The 2.7 kb ubiquitously [illegible] cid phosphoprotein that [illegible] ser activity on promoters that [illegible] ***DNA*** -

protein at the
the C-terminus is reminiscent
of the ets family; however,
ERF and ELK1 or any other
in. The repressor activity of
er ets genes that are known
e, ERF can suppress the
the gag-myb-ets fusion
otein levels remain constant
tion level of the protein
and after mitogenic
yperphosphorylated in cells
d v-src genes and the
F is decreased after co-
-ras or the kinase domain of
ctivity is probably regulated
he in vivo phosphorylation
ently phosphorylated in vitro
sites similar to those
e mutation at position 526
peptide both in in vivo and
on of Thr526 for glutamic
of ERF. Our data suggest
vity is involved in the
ivated during entry into G1
function by the
ily of genes (i.e.gag-myb-ets)
es involved in cell
tumorigenic effects.

L11
AN
TI b the transcription factor
AU A
CS Facultat de Biologia,
NC
SO 0 13) 270 (41) 24385-91.
CY
DT
LA
FS
EM
AB ription factor that is
We present evidence that.

beta gene, i.e. a PU box
domain of PU.1 in B
macrophages represses I-A
of PU.1 was obtained
domain of the
ation domain. Using the gel
bind to the Y box of the I-A
assay, a supershifted DNA
of NFY proteins bind to the
beta gene expression is
domain.

L11
AN
TI ession of GAL4 fusion
tail.
AU E;
CS Hospital, Charlestown
SO 291-8.
CY
DT
LA
FS
EM
AB an cell lines is an
of the proteins encoded
ever, evaluation of the
ficulty in purification of
specific antibodies which
or for immunocytochemistry
usion systems have been
to the expression product
ified using affinity
ts or by immunocytochemistry
ffinity handle
binding* ***domain***
is widely used to construct
n factors to evaluate
the lack of commercially
ther biochemical
has remained difficult. We
an expression vectors,
nds for pM2, Flag, Histidine
of the yeast

cr peptide (consisting of the
.0 product from
tail of six histidines
tes allow both the
GAL4 DNA-binding domain and
ep er ith another insert.(ABSTRACT
N AT

L11

AN 2

TI a the quail PAX6 (PAX-QNR)
promote ns.

AU S Saule S

CS at ire et Moleculaire, CNRS EP56

SO N

al

CY ND

DT al

LA sh

FS it

EM

AB le gene, which plays an
characterized the
complete QNR) gene. In addition to
an revealed several putative
three myb-responsive elements
ding phosphoprotein that
transfection in
e NR promoter with a vector
ess ne resulted in an increase in
NR experiments we identified
within the promoter
-binding domain fused to the
cient in Pax-QNR promoter
can transactivate through a
truncated ***protein***
d ***domain*** was also
These results show that
the myb protein directly as
situ hybridization that
ng neuroretina,
observations suggest that the
pax-QNR/pax-6.

L11

AN